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VOLUME 124

MAMMALIAN HIBERNATION

PROCEEDINGS OF THE FIRST INTERNATIONAL SYMPOSIUM ON NATURAL MAMMALIAN HIBERNATION, MAY 13-15, 1959

Supported by the Office of Naval Research and sponsored by the American Institute of Biological Sciences.

Edited by

CHARLES P. LYMAN
and
ALBERT R. DAWE

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"Think with compassion on the furry
Where they dig their homesteads deep
And feed on the Summer of their bodies
Through the long Winter of their sleep."

R. P. T. COFFIN

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PREFACE

The papers and discussions collected here represent the proceedings of the First International Symposium on Natural Mammalian Hibernation. As such, it is hoped this book will be a milestone in the study of mammalian hibernation. Anyone reading through this volume will realize that there are great gaps in our knowledge and that much of the critical work remains to be done. It is to be hoped that such a realization will spur the interest in this fascinating field.

In violating the primary principle of homeothermism, the hibernators encounter problems which are unique in the mammalian and avian world. The mechanics of this almost purposeful abandonment of the warm-blooded state are a challenge to the investigator, and the means by which the tissues and organs are able to function at such low temperatures are fruitful avenues for further research. These questions cry out for more basic ecology and more sophisticated physiological techniques. A good beginning only has been made in the fundamental biochemistry of the problems involved. These same challenges attracted Claude Bernard and Raphael Dubois in the last century, but after their time there was only sporadic interest in hibernation until a reawakening which started in the European laboratories about twenty years ago. The interest spread to the New World, and the combined advance of our knowledge in this field during the past decade has greatly accelerated. The purpose of the conference was to bring this knowledge together.

To further this purpose, a series of 26 papers were presented during the first two and one-half days of the Symposium, with the senior editor as chairman. On the afternoon of the last day a "Philosopher's Panel" of seven scientists, with the junior editor as moderator, discussed the problem of mammalian hibernation as a whole. These men had attended the presentation of all the papers, with two minor and unavoidable exceptions, but had not concentrated in this precise field of research. Their refreshing point of view is presented after the formal papers. A final period of discussion, in which everyone could participate, follows the Panel.

The Symposium was held at Massachusetts Institute of Technology's Endicott House in Dedham, Massachusetts, from May 13 to 15, 1959. It was sponsored financially by the Office of Naval Research, and held under the auspices of the American

Institute of Biological Sciences (represented at the Symposium by Irvin C. Mohler). Smith, Kline and French Company of Philadelphia generously supplied refreshments at the end of each long day, and we feel sure that all the participants are grateful to them.

There are a number of people to whom we would like to express our personal thanks. Dr. Roger Reid and Captain Bruce Carr encouraged our original idea for this conference, and have been most helpful throughout. Mrs. Regina C. O'Brien coordinated the arrangements for the members, took notes at the meetings, and proof-read and checked the bibliographies of all the papers. Miss Leola Hoffman of the Office of Naval Research at Chicago took stenographic notes of all the discussions and assisted in bringing the transcriptions of the discussions and the panel meeting into their present readable condition. The help of Mrs. O'Brien and Miss Hoffman has been invaluable.

Captain Trent Ruebush of the Office of Naval Research aided in planning the conference, and attended the meetings. His quiet encouragement and sound advice were a never-failing source of help to us. His untimely death in Cairo is a great shock to all of those who knew and admired him.

C. P. L.
A. R. D.

PARTICIPANTS

Dr. E. F. Adolph, Department of Physiology, School of Medicine and Dentistry, University of Rochester, Rochester, New York.

Dr. Eric G. Ball, Department of Biochemistry, Harvard Medical School, Boston, Massachusetts.

Dr. George A. Bartholomew, Department of Zoology, University of California, Los Angeles, California.

Dr. George H. Bishop, Department of Psychiatry and Neurology, Washington University School of Medicine, Saint Louis, Missouri.

Dr. Bayard H. Brattstrom, Department of Biology, Adelphi College, Garden City, New York.

Dr. Mary Anne Brock, Department of Medicine, Harvard Medical School, Boston, Massachusetts.

Dr. Robert W. Bullard, Department of Physiology, Indiana University Medical Center, Indianapolis, Indiana.

Dr. Albert R. Dawe, Office of Naval Research, Department of the Navy, 86 East Randolph Street, Chicago, Illinois.

Dr. William Dawson, Department of Zoology, University of Michigan, Ann Arbor, Michigan.

Dr. H. Arliss Denyes, Department of Biology, Queen's University, Kingston, Ontario, Canada.

Dr. Martin Eisentraut, Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany.

Dr. Kenneth C. Fisher, Department of Zoology, University of Toronto, Toronto, Canada.

Dr. Eugene Flamboe, Department of Physiology, Colorado State University, Fort Collins, Colorado.

Dr. G. Edgar Folk, Jr., Department of Physiology, College of Medicine, State University of Iowa, Iowa City, Iowa.

Dr. Donald R. Griffin, Department of Biology, Harvard University, Cambridge, Massachusetts.

Dr. Raymond J. Hock, Aretic Aeromedical Laboratory, Fairbanks, Alaska. Present address: White Mountain Research Station, University of California, Big Pine, California.

Dr. Bengt Johansson, Cardiological Laboratory, Department of Medicine, Malmö General Hospital, Malmö, Sweden.

Dr. Nikolai I. Kalabukhov*, Institute of Microbiology and Epidemiology of the South-East of the U.S.S.R., Saratov, U.S.S.R. Present address: Chief Post Office, Post Box n. 10, Astrakhan, U.S.S.R.

Mr. Frank C. Kallen, Department of Zoology, Cornell University, Ithaca, New York.

Dr. Charles Kayser, Institut de Physiologie, Faculté de Médecine, Université de Strasbourg, Strasbourg, France.

Mr. Theodore H. Kehl, Department of Zoology, University of Wisconsin, Madison, Wisconsin.

Dr. Barbara R. Landau, Department of Physiology, Saint Louis University School of Medicine, Saint Louis, Missouri. Present address: Department of Physiology, University of Wisconsin, Madison, Wisconsin.

Dr. Basile J. Luyet, Research Laboratory, Department of Biophysics, American Foundation for Biological Research, Madison, Wisconsin.

Dr. Charles P. Lyman, Department of Anatomy, Harvard Medical School, Boston, Massachusetts, and Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts.

Dr. William V. Mayer, Department of Biology, Wayne State University, Detroit, Michigan.

* Not present.

Mr. Michael Menaker, Department of Biology, Princeton University, Princeton, New Jersey. Present address: Department of Biology, Harvard University, Cambridge, Massachusetts.

Mrs. Marion P. Meyer, Department of Zoology, University of Wisconsin, Madison, Wisconsin.

Dr. Peter Morrison, Departments of Physiology and of Zoology, University of Wisconsin, Madison, Wisconsin.

Dr. X. Joseph Musacchia, Department of Biology, Saint Louis University, Saint Louis, Missouri.

Dr. Oliver P. Pearson, Museum of Vertebrate Zoology, University of California, Berkeley, California.

Mr. E. T. Pengelley, Department of Zoology, University of Toronto, Toronto, Canada. Present address: Department of Zoology, University of California, Davis, California.

Dr. Vojin Popovic, Division of Applied Biology, National Research Council, Ottawa, Canada.

Dr. C. Ladd Prosser, Department of Physiology, University of Illinois, Urbana, Illinois.

Dr. Marvin L. Riedesel, Department of Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania. Present address: Department of Biology, University of New Mexico, Albuquerque, New Mexico.

Capt. Trenton K. Ruebush, U.S.N., Physiology Branch, Office of Naval Research, Department of the Navy, Washington, D. C. (Now Deceased).

Dr. H. S. Samuli Sarajas, Institute of Physiology, University of Helsinki, Helsinki, Finland.

Dr. Edward Schönbaum, Department of Surgery, Banting Institute, University of Toronto, Toronto, Canada.

Dr. Douglas E. Smith, Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Illinois.

Dr. Frank E. South, Department of Physiology, University of Illinois College of Medicine, Chicago, Illinois.

Mr. John Steen, Department of Biology, Harvard University, Cambridge, Massachusetts.

Dr. Felix Strumwasser, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland.

Dr. Paavo Suomalainen, Zoological Laboratory, University of Helsinki, Helsinki, Finland.

Mr. John S. Willis, Department of Biology, Harvard University, Cambridge, Massachusetts.

Dr. William A. Wimsatt, Department of Zoology, Cornell University, Ithaca, New York.

Dr. Marilyn L. Zimny, Department of Anatomy, School of Medicine, Louisiana State University, New Orleans, Louisiana.

I

HIBERNATION VERSUS HYPOTHERMIA

By Charles Kayser
Institut de Physiologie, Faculté de Médecine
Université de Strasbourg
Strasbourg, France

If we compare physiological hibernation of mammals with experimental hypothermia of homeothermic mammals, three points appear to be essential:

(1) The difference between homeothermic mammals and active hibernators in summer as to their response to artificial cooling;

(2) The difference between active hibernators in summer and hibernating hibernators;

(3) Despite the differences appearing in these comparisons, a more careful study shows that there are many intermediate states between these extremes. The intermediate states are shown by the study of the effect of climatic factors on certain active hibernators in summer, and by the study of the incomplete homeotherms, especially those from the Southern Hemisphere.

This special point will not be considered, Dr. Morrison being more informed than I.

First Point

A) Since Walther (1865) and his pupil Horvath (1876), we know the essential differences which contrast experimental hypothermia in active hibernators in summer with that in homeothermic rodents. These differences are:

(1) Artificial cooling (immersion in cold water) produces death by respiratory arrest in homeothermic animals when the central temperature falls to 19°C; the same method fails to stop the respiration of hibernators down to central temperatures of about 5°C.

(2) The use of artificial respiration allows the lowering of the central temperature of homeothermic mammals down to about 10°C.

(3) The speed of cooling, in cold water, is much faster for the ground squirrel than for the rabbit.

(4) The rabbit's death in hypothermia is to be ascribed to some encephalic vascular trouble, as the eye fundus appears slate-colored.

These four observations show the importance of the special resistance of the nervous system of hibernators towards deep hypothermia. Hall (1832), Dubois (1896), Merzbacher (1904), and Uiberall (1934) all emphasized the importance of the nervous system in hibernation. Lyman and Chatfield (1950, 1953), Kayser *et al.* (1951), and recently Strumwasser (1959a,b) also pointed it out. Chatfield *et al.* (1948) could show the persistence of nervous conductivity down to 3°C in *in vitro* experiments.

The question arises as to whether it is possible to show a special cellular property which would explain this resistance. We hoped to obtain such evidence by the investigation of the effect of temperature on the respiration of brain slices with Warburg's method; we had observed (1954a,b) that the critical heat increment of the respiration of kidney slices was significantly lower (11,150 gm. cal.) in the European hamster than in the albino rat (13,320 gm. cal.), the hamsters having been killed either in the hibernating or in the active state.

We could, however, find no difference in the critical heat increment (studied between 38°C and 5°C) for the respiration of brain slices between rats and European ground squirrels or European hamsters (Table I).

TABLE I

Critical heat increment of the oxygen consumption of brain cortex slices of albino rat, albino mouse, European hamster and European ground squirrel
(Suspension liquid: Krebs II + glucose, without lactate, pyruvate or fumarate).

Species Physiological state	Number of animals	Number of measurements	Critical heat increment (gm. cal.)	Correlation coefficient (r)	Mean body weight (gm.)
Albino rat	18	27	13,483	0.979	199
Albino mouse	12	12	12,205	0.985	24
European hamster active (summer)	12	13	13,611	0.986	263
European hamster active (winter)	13	16	13,051	0.914	285
European hamster hibernating (winter)	13	19	13,326	0.972	270
European ground squirrel, hibernating (winter)	5	5	13,722	0.985	157

This observation led us to study systematically the critical heat increment for the oxygen consumption of all the tissue slices we could obtain, without any major difficulty, in hibernators and homeothermic mammals (Table II, A and B).

TABLE IIA

Critical heat increment of the oxygen consumption of slices of various tissues of three homeothermic rodents (guinea pig, albino rat, albino mouse).
(Suspension liquid: Krebs II + glucose, without lactate, pyruvate or fumarate).

Species	Mean body weight (gm.)	Number of animals	Number of measurements	Tissue	Critical heat increment (gm. cal.)	Correlation coefficient
Guinea pig	846	7	15	Kidney	14,292	0.961
Albino rat	193	13	26	Kidney	13,319	0.984
" "	199	18	27	Brain cortex	13,483	0.979
" "	193	21	39	Liver	13,918	0.974
" "	178	13	17	Spleen	14,345	0.906
" "	191	16	17	Heart muscle	8,853	0.878
Albino mouse	19.6	12	20	Kidney	12,668	0.959
" "	24.0	12	12	Brain cortex	12,205	0.985
" "	22.9	10	15	Liver	12,068	0.961
" "	21.0	9	11	Spleen	14,586	0.973
" "	20.0	8	8	Lung	11,467	0.961
" "	21.7	13	16	Heart muscle	8,861	0.978
" "	24.0	26	26	Diaphragm	7,165	0.968

TABLE IIB

Critical heat increment of the oxygen consumption of slices of various tissues of three hibernators (European hamster, European ground squirrel, marmot) in different physiological states.
(Suspension liquid: Krebs II + glucose, without lactate, pyruvate or fumarate).

Species Physiological state	Mean body weight (gm.)	Number of animals	Number of measurements	Tissue	Critical heat increment (gm. cal.)	Correlation coefficient
European hamster active (summer)	254	10	20	Kidney	11,151	0.991
"	263	12	13	Cerebral cortex	13,611	0.986
"	271	9	9	Liver	15,981	0.958
"	270	6	11	Heart muscle	7,878	0.943

TABLE IIB (Continued)

Species Physiological state	Mean body weight (gm.)	Number of animals	Number of measurements	Tissue	Critical heat increment (gm. cal.)	Correlation coefficient
European hamster active (winter)	293	9	20	Kidney	13,302	0.982
"	285	13	16	Cerebral cortex	13,051	0.914
"	308	13	27	Liver	11,168	0.902
"	260	6	6	Heart muscle	8,414	0.900
European hamster hibernating	242	12	25	Kidney	11,727	0.988
"	270	13	19	Cerebral cortex	13,501	0.972
"	298	13	26	Liver	13,326	0.945
"	226	8	8	Spleen	11,216	0.947
"	241	10	17	Heart muscle	10,792	0.927
European ground squirrel, active (summer)	154	5	5	Kidney	14,125	0.987
"	156	5	5	Liver	10,498	0.967
European ground squirrel, hibernating (winter)	151	11	11	Kidney	11,084	0.939
"	157	5	5	Cerebral cortex	13,722	0.985
"	154	12	23	Liver	11,113	0.939
"	159	6	6	Spleen	12,588	0.984
"	159	6	10	Lung	9,901	0.949
Marmot, active (summer)	3,000	2	7	Kidney	14,448	0.983
"	3,000	2	7	Liver	11,388	0.976
Marmot, hibernating	2,357	3	11	Kidney	12,227	0.999
"	2,357	3	11	Liver	11,658	0.993

There appeared to be no systematic difference as to the effect of temperature on tissue respiration between homeotherms (albino rat, albino mouse, guinea pig) and hibernators (marmot, ground squirrel, hamster).

This observation seems normal: we know today that temperatures near 0°C do not kill the tissues of homeotherms.

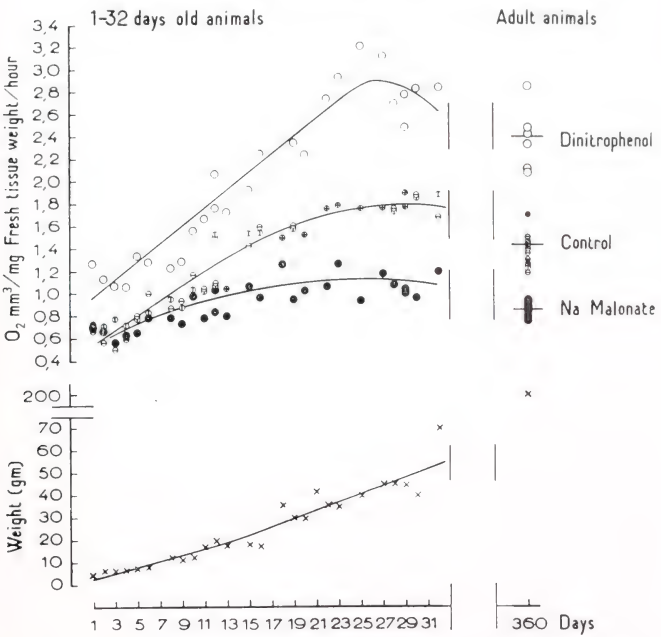

Fig. 1. Effect of Na malonate (0.01 M) and dinitrophenol (10^{-6}M) on the oxygen consumption of brain slices of growing rats (Kayser and Lucot, 1959).

After this failure, we approached the same problem in another way; like Tyler (1942), Chesler and Himwich (1944), and Locker (1958), we decided to make use of sodium malonate and dinitrophenol in respiratory experiments *in vitro*. We were able to confirm the observations of Tyler: malonate depresses the oxygen consumption of brain slices from new-born rats less than it does in the case of adult ones (Fig. 1).

We observed no variation in respect to age in the effect of dinitrophenol on the oxygen consumption of brain slices from rats.

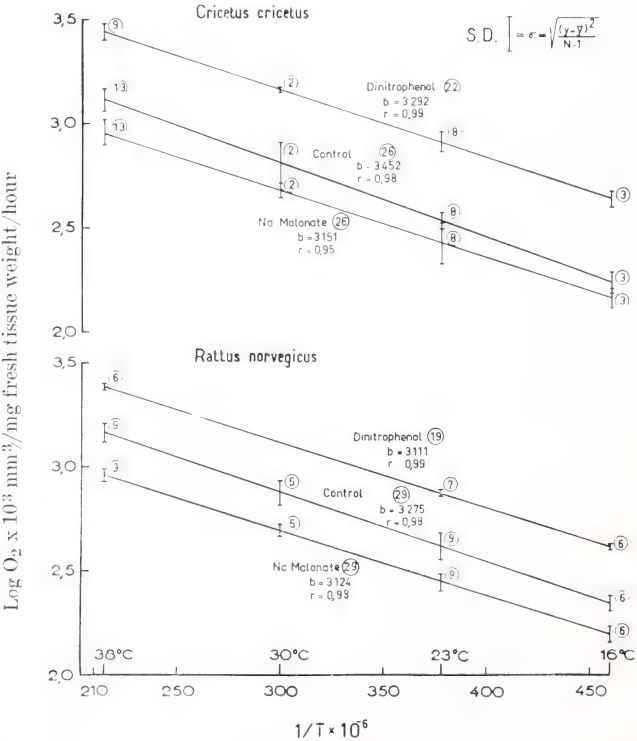

Fig. 2. Critical heat increment for the respiration of brain slices from European hamsters and albino rats under the action of Na malonate (0,01 M) and dinitrophenol (10^{-6}M) (Kayser, 1959a). Encircled numbers = number of animals used.

The use of the same poisons in respiratory experiments on brain slices from rats or hamsters shows that the depressing

effect of Na malonate is less in hamsters than in rats, and that dinitrophenol increases the oxygen consumption more (120 per cent) in hamsters than in rats (70 per cent). The differences observed are statistically valid (Fig. 2).

We think that the observations of Edwards (1824), Britton and Kline (1945), Adolph (1948, 1951), Adolph and Lawrow (1951) and Hiestand *et al.* (1950) on the resistance of young mammals and hibernators to anoxia and hypothermia could be explained by the fact that glycolysis plays a more important role in the energetics of the brain of young homeothermic mammals and hibernators than in adult homeotherms.

B) It is well-known that the variations of nervous excitability with temperature depend to a large extent on the Ca/K ratio: if K increases in the suspension liquid, the nerve block appears at a higher temperature and if K decreases the block appears at a lower temperature. If there is an increase in Ca concentration, the nerve block appears at 0−2°C instead of 10°C.

Studying the length of the different phases of the EKG we had observed (Kayser, 1957a) that the effect of temperature on the distance between the S inflection and the summit of the Osborn-wave (Osborn, 1953) in cooled homeotherms was nearly null (critical heat increment of 1,000 gm.cal. approximately). The effect of temperature on the same distance in cooled hibernators showed the same heat increment as for the oxygen consumption of heart muscle slices (7,000-8,000 gm.cal.). The value of 1,000 could not be statistically ensured. We were then unable to assert that the effect of temperature on the repolarization was different in cooled homeotherms and in cooled hibernators.

Recently, G. Bach (personal communication, 1959) repeated the same experiments with cooled dogs and observed the same value for the critical heat increment for S-O distance (7,000-8,000 gm.cal.) as we had observed in cooled hibernators (Table III).

TABLE III

Critical heat increment of the quick repolarization phase of the heart muscle

Species	Critical heat increment (gm. cal.)	Author	Correlation coefficient	Number of measurements	Number of animals
Dog	8,143	Bach	0.64	43	5
European hamster	7,843	Kayser	0.74	57	5
European ground squirrel	7,093	Kayser	0.81	39	4

Second Point

The difference between active hibernators in summer and hibernating hibernators appears with much sharpness if we compare the basal metabolic rate of active hibernators in summer with the minimal heat production of the same species during deep hibernation (Fig. 3).

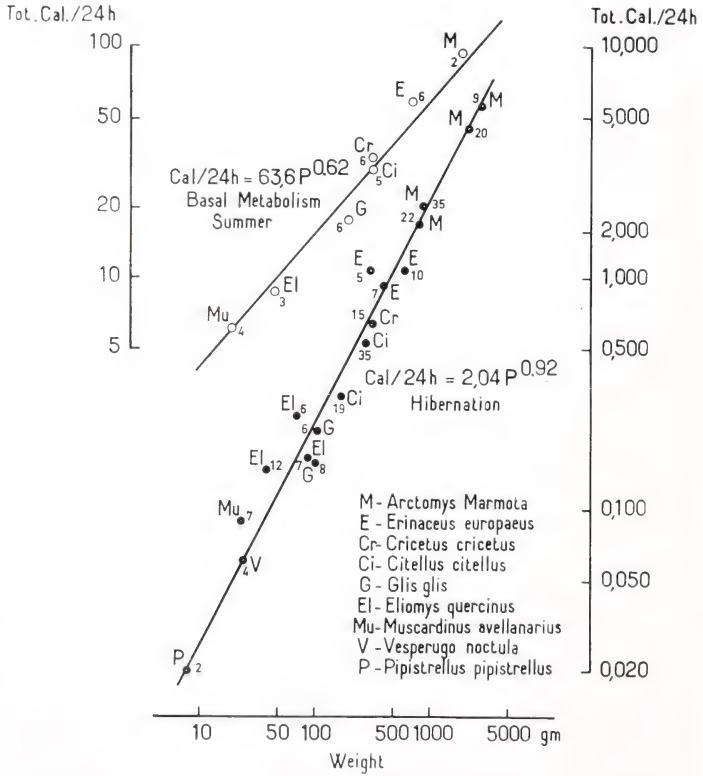

Fig. 3. Basal metabolic rate of some active hibernators in summer and hibernating hibernators (Kayser, 1959b).

In the active state, the surface law accounts for the heat production but in the hibernating state the surface law disappears and the heat production is the same per unit of body weight for the marmot of 2.5 kg. and the bat of 5 gm.

The disappearance of the surface law in the hibernating state is to be related to the special endocrine syndrome in hiber-

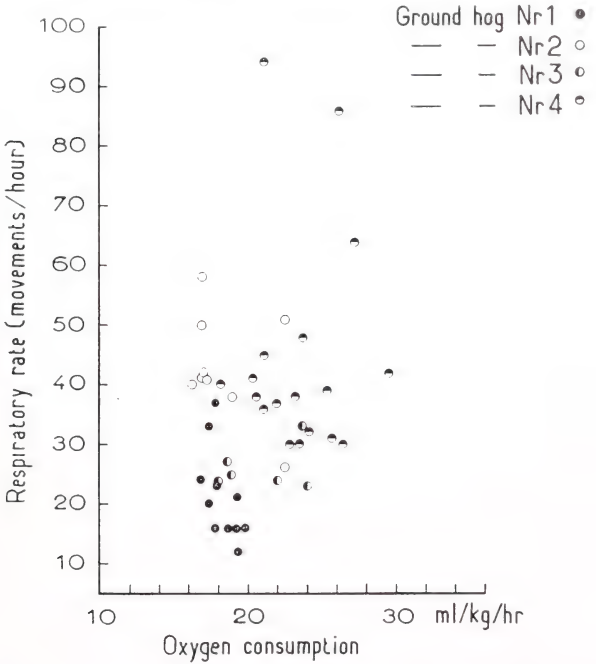

Fig. 4. Respiratory frequency versus oxygen consumption in hibernating marmots (Kayser, 1940a).

nation and the special functioning of the nervous system in this state: Galvao (1947, 1948-49, 1950-51) shows the disappearance of the surface law in dogs and men acclimatized to tropical climate, and Brendel and Usinger (personal communication, 1959) find this also in the deep hypothermia of narcotized dogs, cats and rabbits.

The special functioning of the nervous system in hibernation is shown if we study the respiratory frequency versus the oxygen consumption in six-hour experiments on hibernating marmots (Kayser, 1940a) (Fig. 4), or the heart frequency versus the respiratory frequency in hibernating European ground squirrels (Kayser, 1957b) (Fig. 5).

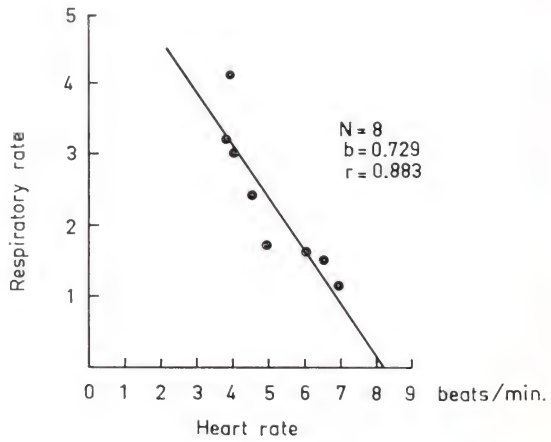

Fig. 5. Heart frequency versus respiratory frequency in hibernating European ground squirrels (Kayser, 1957b).

Figure 4 shows that there is no correlation between respiratory frequency and oxygen consumption, and Figure 5 shows that there is a negative correlation between heart frequency and respiratory frequency in deep hibernation. Both functions, respiratory and cardiac, remain under the influence of the nervous system but there is no longer integration of these two functions in respect to the energetic needs in hibernation.

Third Point

Despite these enormous differences which contrast a hibernating hibernator to itself in the active state in summer, and which

contrast it in artificial cooling experiments to a cooled homeotherm, there are as many intermediate states between hibernators like bats and poikilotherms.

All the authors who, as Shaw (1921, 1925a,b), Johnson (1930), Ismagilov (1955) and others, assert that climatic factors intervene in the entrance into hibernation, implicitly emphasize that hibernation — a true regulation to a minimal energetic expenditure — is also the effect of the overstraining of the animal. We arrive thus at the conception of Suomalainen and Nyholm (1956) that hibernation is also an adaptative syndrome of Selye.

In 1958 we detected in summer, in a small hibernator, the garden dormouse (50 gm), states of hypothermia very near to true hibernation (Kayser *et al.*, 1958; Lachiver and Kayser, 1958). These states were obtained by maintaining the animals at 5-7°C with or without nest-building material and food. We obtained in this way states of acclimatization with increased heat production. As in our experiments in 1939 (Kayser, 1939b), the oxygen consumption measured in the afternoon in experiments at 5°C was above 5,000 ml/kg/hr. But we also observed states of deep hypothermia (central temperature below 10°C) in the morning (Table IV).

TABLE IV

Frequency of deep hypothermia in garden dormice, in August. The animals remained at +5°C environmental temperature.

	Animals fed	Animals having starved for 24 hr	48 hr	72 hr
Number of hypothermic animals	6	17	17	10
Number of normothermic animals	62	2	0	0

From Lachiver and Kayser (1958).

After 48 hours starvation, one hundred per cent of the animals are in deep hypothermia. The combined effect of cold and starvation induces hypothermia. This hypothermia is only an accentuation of the diurnal rhythm: in the evening the animals arouse: if they have the opportunity to feed, they keep their normal body weight; if they are starved, the body weight decreases by 9 per cent during the first 24 hour starvation period, 5 per cent from the second to the third day, and 2 per cent the last day.

This weight loss is very important, but in two dormice starved in June for 8 days (the animals remaining untouched for this time) the weight loss was 0.7 per cent per day, a value very near to that observed in deep hibernation (0.6 per cent).

The oxygen consumption and the heart frequency in deeply hypothermic dormice were very inconstant (Table V).

TABLE V

Oxygen consumption, heart frequency and colonic temperature of 5 garden dormice staying at +5°C in August.

Number of measurements	Number of animals	Mean length of measurement (min.)	Mean body weight (gm.)	O_2 consumption (ml/kg/hr)	Mean colonic temperature at the end of the measurements (°C)	Mean heart frequency at the end of the measurements (beats/min.)
3	3	76 (±32)	50.2 (±2.4)	64.1 (± 12)	5.7 (±0.6)	27 (± 3)
2	2	87 (±47)	50.2 (±0.7)	150.8 (± 34)	5.9 (±0.1)	27 (± 0)
8	4	59 (±24)	52.1 (±2.6)	627.8 (± 99)	7.7 (±0.9)	65 (± 8)
6	3	57 (±29)	56.3 (±3.3)	1,528 (±256)	11.3 (±5.0)	94 (±18)
2	1	46 (±10)	54.5	2,840 (±506)	12.0 (±0.5)	330 (one measurement)

From Kayser, Lachiver and Rietsch (1958).

If we study the animals from August to December (Table VI), we see that the minimal oxygen consumption falls from 64.1 (August) down to 39 (October), 28.5 (November) and 31.1 (December) (Tables V, VII).

TABLE VI

Hypothermia and hibernation in garden dormice staying at 5-7°C from August to January (animals in individual cages, fed and with nest-building material at their disposal).

Month	Environmental temperature (°C)	Number of animals	Number of observations	Number of active states	Number of hypothermic states	Torpidity (%)
August	5	6	68	62	6	8.8
September	7	4	84	79	5	5.9
October	5	4	112	35	77	68.6
November	5	5	116	13	103	88.7
December	6	5	55	4	51	92.7

From Lachiver and Kayser (1958).

The frequency of the hypothermic states in fed animals increases sharply in October: we pass from hypothermia to hibernation (Table VII).

TABLE VII

Oxygen consumption of 4 garden dormice, staying at +5°C from September to January (animals in individual cages, fed, and with nest-building material at their disposal).

Month	Number of measurements	Number of animals	Body weight (gm)	Oxygen consumption (ml/kg/hr)	Length of measurement (min.)
October	14	2	54 (± 3.0)	39.0(± 4.7)	201
"	6	2	55.3(± 4.8)	55.8(± 4.1)	200
"	3	2	70.2(±11.1)	280.8(±97.8)	225
November	5	2	88.9(± 1.3)	28.3(± 5.1)	193
"	6	2	95.7(± 8.9)	79.3(± 8.6)	194
"	5	2	95.0(± 7.2)	354.5(±77.7)	194
December	8	3	72.3(± 6.1)	31.1(± 4.9)	194
"	5	3	75.8(± 8.9)	59.3(± 5.9)	183

From Kayser, Lachiver and Rietsch (1958).

The study of the thyroid glands, removed in August or in December or January on the first day of the experiment, shows a hyperactivity in August, and a reduced activity in January or December, but the degree of involution is very variable (Plate).

The conclusion is evident: staying at a low temperature produces, in small hibernators, states very near to true hibernation in spite of very active thyroid glands. These deep hypothermic states are, at the beginning, only an accentuation of the normal diurnal rhythm of temperature; the "internal clock" still works: in the evening the animal arouses. In deep hibernation we no longer observe a difference between the oxygen consumption from 9 a.m. to noon and that measured from 3 p.m. to 6 p.m. (Table VIII).

TABLE VIII

Oxygen consumption of hibernating garden dormice (December/January), staying at +6°C (two measurements on each day, regularly, on the same animal, from 9 to 12 a.m. and from 3 to 6 p.m.).

	Number of measurements	Oxygen consumption (ml/kg/hr)	Standard deviation
Morning	16	38.87	12.3
Afternoon	16	42.53	22.1
		(t = 0.57)	

However, all conditions remaining constant, the same animals again show the normal diurnal rhythm spontaneously in February, at the end of hibernation: the frequency of arousals is much greater in the evening; the "internal clock" works again.

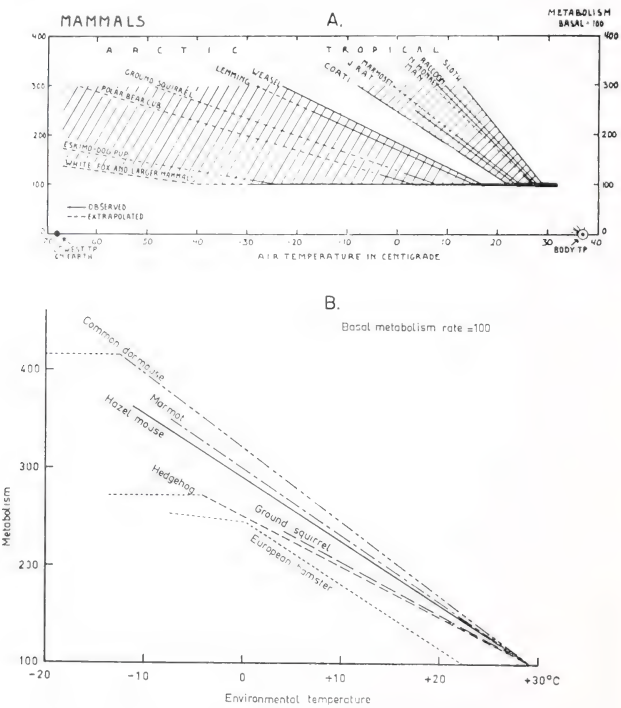

Fig. 6. Increase in heat production by tropical adapted and Arctic adapted animals (A), and by some hibernators (B).

We have asserted already (Kayser, 1940b) that hibernation is realized by the double effect of internal rhythm and external factors. But, if external factors are able to cool active hibernators, it is only possible because hibernators show a deficient physical thermal regulation and that, in autumn the chemical

regulation also decreases (Kayser, 1939a). We insisted on this point and reported the important increase in heat production obtained in summer in active hibernators by lowering the environmental temperature. We compared the heat production of hibernators in summer with the heat production of clipped rabbits and of rabbits with thoracic medullary section. The increase of heat production per degree of lowering of the environmental temperature was 6.0 per cent. This observation is in opposition to the observations of Scholander *et al.* (1950), Erikson (1956) and Irving (1958) (Fig. 6).

The values related for the Arctic ground squirrel by Scholander *et al.* (1950) are for the most part extrapolated, and the values really measured by Erikson (1956) on the same animal at −20°C are much higher. But Erikson affirms that there is no increase in the heat production of quiet Arctic ground squirrels between +30° and +5°C. This seems to be real, but I think that the quiet animals studied at 5°C by Erikson were hypothermic. Erikson himself, like other physiologists, reports the abnormally accentuated diurnal rhythm in oxygen consumption in the Arctic ground squirrel. Such a lowering of oxygen consumption during sleep cannot be understood in such a perfect homeotherm as the animal described by Scholander.

It is difficult to understand why such a perfect homeotherm would become hypothermic and hibernate, if its peak metabolism is observed at temperatures near to the lowest temperatures recorded at the coldest places on the earth.

General Conclusions

It is evident that the experimental hypothermia of the homeothermic animal fundamentally differs from hibernation, as the functioning of the nervous system of the awake and active hibernator is different from that of the homeotherm; it is also evident that a hibernating hibernator may not be confused with a hibernator made hypothermic in summer by the suppression of its thermoregulation, and that hibernation appears, then, as a regulation to a minimum level recorded only in certain well-defined species; it is not less obvious that between the extremes there are intermediaries: the small-sized true hibernators — bats and garden dormice in the active state in summer — supply us with a first example.

The states of dormancy recorded in prosimians (Bourlière, Petter and Petter-Rousseaux, 1956), in marsupials (Coleman,

1938; Fleay, 1944; Bourlière, 1951), in the female bear (Matson, 1946; Lobatchev, 1956; Hock, 1958), and in the raccoon (Sharp and Sharp, 1956) are other examples.

The accidental hypothermia of certain birds (hummingbirds, swallows) is still another example (Dupond, 1937; Huxley *et al.*, 1939; Koskimies, 1948; Bartholomew *et al.*, 1957); the birds "adapt" themselves to hypothermia.

These hypothermic states (the examples could be multiplied) are withstood, if deep, but for rather short times: in our experiments on hamsters they could not go beyond 48 hours, unless the animal was rewarmed every other day (Kayser, 1955). If the hypothermia stabilized itself at about 30°C (black bear), it proceeds as the hypothermia of 5°C of a true hibernator of the Northern Hemisphere.

But in hibernation also a hypothermia of several months cannot occur: in the ground squirrel, the longest durations we have observed were of 35 to 40 days. If, in the garden dormouse, they went beyond 25 days, death was the almost unavoidable consequence. It is our belief that only the poikilothermic animals may hibernate for months without any thermal ascension being necessary.

Thus, studied in a single species, such as the garden dormouse or the bat, true hibernation may show analogies with the hypothermia of hibernators in summer. Studied in the whole animal scale, hibernation places itself between the "Winterstarre" or winter rigidity of Eisentraut (1933) and the "Winterruhe" or dormancy, and from the dormancy one may pass to the states of accidental hypothermia. If the extreme cases are sharply defined, we must, however, make allowance for the intermediaries.

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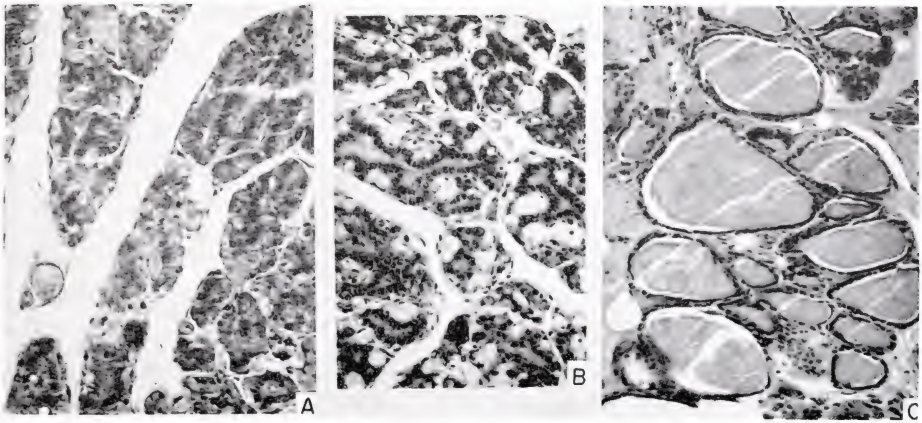
DISCUSSION FOLLOWING KAYSER'S PAPER

Two questions were raised (the first by BISHOP, the second by SOUTH) which attempted to specify more particularly differences observed in the uptake of oxygen by the brain slices. In the first case, the notion was advanced that brain or nerve has two "kinds" of metabolism, one seen in the resting state, and one in activity, and that KAYSER may have been actually measuring this difference rather than a difference truly associated with the active versus the hibernating state. The second question cast doubt on the use of competitive inhibitors such as malonate, since malonate (as a competitive inhibitor) might be more active at a lower temperature and hence modify the results (rather than the effect noted being strictly physiological).

In the first instance, KAYSER was not aware of any experiment which would affirm that there would be any difference in sign of activity or of rest in brain slices from awake animals (rats or hamsters) killed by neck section, and in the second instance he stated that he had seen no difference in the critical heat increment of the oxygen consumption of brain slices in the presence or absence of malonate either in the hamster or rat.



A) Thyroid from August 31, 1958 B) Thyroid from January 1, 1959 C) Thyroid from January 1, 1959

Plate. Thyroid glands from hypothermic dormice staying at 5°C from August to January.

II

HEAT REGULATION IN PRIMITIVE MAMMALS AND IN TROPICAL SPECIES

By Martin Eisentraut
The Alexander Koenig Institute
for Zoological Research and Museum
Bonn, Germany

The state of hibernation into which some homeothermic animals fall during the period of unfavorable external conditions is characterized, among other concomitant phenomena, by the behavior of the body temperature. A hibernator is capable of becoming cold just like a poikilothermic animal. In this case, however, there is not attained a complete elimination of the heat-regulating arrangements, but merely a change-over, an adjustment of the regulation to a lower stimulus threshold. This indicates to us that the problem of hibernation is essentially a problem of heat regulation and heat economy.

The varying level of development of heat economy in animals quite generally is shown in the relation of the body temperature to the outside temperature (Fig. 1). The poikilothermic animal is to a large extent dependent on the warmth of the environment. If this decreases, the body cools thoroughly and the animal in question gradually falls into a state of torpidity. Many poikilothermic animals of the temperate zones, for example, some insects, can be sub-cooled in the winter below 0°C, without freezing of the body fluids. When a critical point is reached, the so-called sudden transition of temperature occurs: the body suddenly heats up to almost 0°C, and not until then does congealing of the body fluids ensue, and the cooling ends in death.

The homeothermic animal, on the other hand, is able to maintain its body temperature at a level which is optimum for it, independent of the temperature of the environment. As the external temperature drops, the homeothermic animal combats the cooling of its body by increased metabolism, by motion, and by other precautionary measures. In this way it counterbalances the heat loss by increased heat production. Thus, for example, homeothermic animals of the polar region can withstand very considerable degrees of cold without harm. Other homeothermic animals lack such a power of resistance against cold. With long or intense action of cold the resistance disappears and a hypothermia comes about. If in this process the temperature drops

below a certain level, which varies with the different species, the normal nerve function is blocked; respiration and heart-action stop. In this way the hypothermic state likewise leads to a lethal end.

This is different in the case of the hibernator, provided that it has the internal predisposition toward hibernation which may be caused by hormones. If the environmental temperature drops to a critical point, which is a characteristic one for the individual species, the hibernator puts its regulating mechanism out of

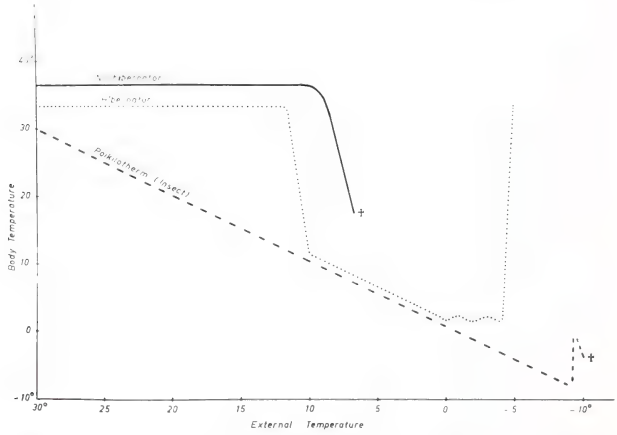


Fig. 1. Schematic curve course of the body temperature versus the external temperature.

circuit and becomes cold. Its body temperature approximates the environmental temperature more or less, as in the case of a poikilothermic animal. Not until the minimum temperature, which lies around 0°C, is approached, does the regulating mechanism become active again; as a matter of fact, this is attributable to the sensitivity, which is maintained even at low temperatures. There comes about an increase of the metabolism, and with that a production of heat, which under certain circumstances can lead to an awakening from the lethargy of hibernation. This is an essential property of hibernators and one which distinguishes them from non-hibernating homeothermic and poikilothermic animals.

In respect to these peculiarities of heat regulation, it is quite generally of interest to consider the heat economy in various species of mammals, from a comparative standpoint. Here we can begin with the assumption that in the course of phylogeny homeothermism has developed from poikilothermism. This process is once more briefly repeated during the ontogeny of the individual: the young mammal, for example, a mouse, does not acquire the ability to maintain a temperature of its own, independent of the environmental temperature, before the post-embryonic nestling period.

Not all homeothermic animals have attained the highest degree of perfection in respect to their heat regulation. Close investigations have demonstrated that, especially, phylogenetically old mammals are often still characterized by a very primitive heat economy. Formerly this was occasionally designated as heterothermism and was separated from the homeothermic and poikilothermic animals as a third group. I do not consider this tripartite division as a very fortunate choice, but I distinguish between *higher* and *lower* warm-blooded animals, which are contrasted to the cold-blooded ones as a unit (Eisentraut, 1953). In this case, of course, it must not be expected that these two groups of homeothermic animals can be sharply separated from each other. Rather, it is more in keeping with our concept of phylogenetic development to find that there is a smooth transition from the one into the other.

First, I will single out only a few examples: man, among others, belongs to the higher warm-blooded animals with a very perfect heat regulation. In man, the rectal temperature varies in the daily rhythm from about 36.7°C early in the morning to 37.5°C late in the afternoon. The Carnivora and the Ungulata are likewise highly developed warm-blooded animals. In the dog and the cat, the range of variation amounts to about 2°C and extends from 37.5°C to 39.5°C. The horse and the bovine show temperatures between 37.5°C and 38.5°C, hence a range of variation of only one degree (Eisentraut, 1956b). Higher warm-blooded animals, therefore, have a relatively high average temperature independent of the environmental temperature, and a relatively slight range of variation of their activity temperature occurs within the daily rhythm.

In contrast to this, among the lower warm-blooded animals the average temperature is relatively low and the range of variation of the activity temperature is often, but not always, relatively large. The Madagascan Tenrec, *Centetes caudatus*, is a typical

example of a lower warm-blooded animal. I shall return to a consideration of it soon.

Before we discuss further examples in detail, it seems important to define briefly the individual temperature ranges. The range of activity embraces *all* temperatures which an animal shows during the waking state *and* during dormancy. In this euthermic state the animal is at all times capable, without restraint, of making use of all its motor and sensory functions. If the body heat sinks below the lower limit of the range of activity, that is, if it drops below the activity threshold, it reaches the hypothermic range of lethargy. Correspondingly, if the upper limit of the activity range is exceeded, we can speak of hyperthermic temperatures.

The data and the temperature measurements available for mammals up to now have shown that, in general, higher warm-blooded animals have an activity temperature above 36°C, while in lower warm-blooded animals it lies, on the average, below 36°C.

The following compilation (Fig. 2) offers a series of further examples of activity temperatures in mammals. Here, it is a question partly of species which I was able to examine myself, and partly of data from the literature, only a few of which, to be sure, can be used for our purposes.

Of the higher warm-blooded animals (I) there are considered here only the previously-mentioned representatives: man, cat and bovine. In this group, there might be mentioned also a whole series of lagomorphs and rodents, such as rabbits, guinea pigs and rats. The great order of Rodentia, which has very extensive ramifications, however, also includes representatives which pass over into the realm of the lower warm-blooded animals on the basis of their very labile heat economy.

Of the lower warm-blooded animals listed in Figure 2 (II), primarily species are mentioned which we must consider old from a phylogenetic standpoint: of the monotremes *Tachyglossus aculeatus* (Sutherland, 1897; Wardlaw, 1915); of the marsupials *Marmosa cinerea* (Eisentraut, 1955) and *Metachirus nudicaudatus* (Morrison, 1946); of the insectivores *Centetes ecaudatus* (Eisentraut, 1955), *Hemiechinus auritus*, and *Paraechinus aethiopicus* (Eisentraut, 1952, 1956a); from the order of the Xenarthra the sloth *Bradypus griseus* (Britton and Atkinson, 1938), and the armadillos *Tolypeutes conurus* and *Tatus novemcinctus* (Eisentraut, 1932a,b), and of the scaly anteaters *Manis tricuspis* (Eisentraut, 1956c).

In most of the lower warm-blooded animals considered here, in all of which the average level of the activity temperature lies below 36°C, their great range of variation is striking. It is most pronounced in the Madagascan hedgehog *Centetes ecaudatus*. I had the opportunity to examine quite closely and to measure

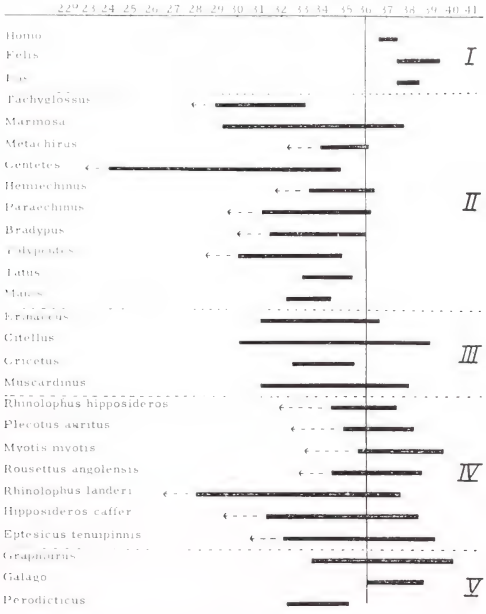


Fig. 2. Range of activity temperature in different mammals. Temperature in degrees Centigrade.

the body temperatures of two specimens of this species, which probably stands on the lowest stage of the placental or monodelphic mammals (Eutheria). The range of variation amounts to more than 10°C, from 24.1° to 34.8°C. I should like to state in this connection that the high values are reached only with very intensive activity, and the low values after very long dormancy. When the animal is aroused from its deep sleep, it is still able, in the lowest range of the activity temperature, to

react spontaneously to external stimuli, to move normally, to drink and to ingest food. Only with body temperatures from 24°C on down does a lethargy, and with it an inhibition of the bodily functions, ensue.

In Figure 2, accordingly, the continuous lines indicate the range of variation of the activity temperature, which occurs for each species in the daily rhythm. We frequently find, however, among the lower warm-blooded animals, in addition to this, a dependence of the body temperature on the level of the environmental temperature. If the latter drops off very much, some animals, for example, *Tachyglossus*, *Centetes*, and two species of hedgehog, *Paraechinus* and *Hemiechinus*, and also *Bradypus* and *Tolypeutes*, incline toward hypothermia. I have indicated this phenomenon by dotted lines. Conversely, with very high environmental temperatures, the body temperature can exceed the upper limit of the normal range of activity, as shown, among others, by the observations made by Morrison (1946) on *Metachirus* and *Didelphis*. I do not intend, however, to enter into the details of these hyperthermic phenomena here.

We can, therefore, state quite generally that the lower warm-blooded animals are characterized by a very labile heat economy and a primitive regulatory mechanism.

Of the species mentioned, *Tachyglossus* and the three representatives of the Insectivora belong to the hibernators or, at any rate, to the species which are able to undergo at least a voluntary hibernation. With this we come to the consideration of the hibernators themselves. In Figure 2 (III), the activity ranges of the body temperature outside of the hibernation period are indicated for a few of the best known representatives. In their case, too, it strikes one's attention that the range of variation of the normal body temperature is very broad. Thus, for example, in the European hedgehog, *Erinaceus europaeus*, the activity range of the body temperature extends, according to Groebbels (1926) and according to my own observations (Eisentraut, 1956a), from 31.1° to 36.7°C. According to Wade (1930), *Citellus tridecemlineatus* even has a range of variation from 30° to 39°C. In the hamster, *Cricetus cricetus*, I found a range from 32.5° to 35.5°C (Eisentraut, 1928). This imperfection in heat regulation among hibernators has been stressed frequently in the literature, and, on the basis of this peculiarity, we will probably have to include also all known hibernators in the group of the lower warm-blooded animals.

The order of the Chiroptera (Fig. 2, IV) assumes a special position among the mammals in respect to heat economy. As we know, the representatives of this order inhabiting the temperate zones are hibernators. Concerning the flying foxes (Megachiroptera), which are distributed through the tropics and subtropics of the Old World, only few data are available concerning the level of the heat economy. In the flying fox, *Rousettus angolensis*, with habitat in tropical Africa, I was able to show an activity temperature from 34.4° to 38.6°C (Eisentraut, 1940). With protracted action of cold it is possible to go below the lower threshold of activity, and a hypothermic lethargy then ensues.

For the representatives of the Microchiroptera with habitat in the temperate zones, a very striking lability of the body temperature was shown. The range of activity, which is very hard to define accurately in this case, is relatively large. In *Rhinolophus hipposideros*, for example, it extends from 34.4° to 37.4°C, in *Plecotus auritus* from 35° to 38.2°C, in *Myotis myotis* from 35.6° to 39.6°C (Eisentraut, 1934), but it is possible to drop below this range of activity even during normal day sleep. A state of lethargy is then reached, which I designate as "day-sleep lethargy," and from which the animals awaken in the evening. In this case the body temperature rises again up to the activity range, starting from the inside and proceeding outward. I should like, however, to stress explicitly that during dormancy it is by no means inevitable that a dropping of the body temperature below the activity threshold must produce a lethargy. Everyone who investigates the sleeping quarters of bats during the warm season of the year makes the observation that the animals in question very frequently, even in a relatively cool environmental temperature, are immediately ready for flight and fly away, and hence are not lethargic. But it does also occur in cool weather that some animals are in a hypothermic and lethargic state and can be seized with one's hand without difficulty. Animals kept in confinement very often show day-sleep lethargy. For the bats themselves the strong inclination toward poikilothermism is by no means a disadvantageous phenomenon, but can be of a certain usefulness in the lives of the animals, for through the reduction of metabolism connected with hypothermia the animal saves fuel in the form of insect food.

The demonstration of the primitive heat economy in bats of the temperate zone, which, as has been mentioned, are hibernators, gave rise to an investigation also of purely tropical representatives of this order of mammals in respect to their heat regu-

lating capacity. The observations made by me in the Cameroons (West Africa) (Eisentraut, 1940; 1956c) on Rhinolophides and Vespertilionides likewise indicated a very high range of variation of the activity temperatures. In this case it was shown that the lower limit of the range of variation lies considerably lower than in the representatives adduced for the temperate zone. In *Rhinolophus landeri* the activity range extends from 28° to 37.6°C, in *Hipposideros caffer* from 31.4° to 38.4°C, and in *Eptesicus tenuipinnis* from 32.2° to 39.2°C. Beyond this, however, with sufficiently low environmental temperature — in experiments conducted in a refrigerator — a drop of the body temperature into the hypothermic range occurred even in these tropical species. A few more detailed data on this follow:

In *Hipposideros caffer* the body temperature can drop below the activity threshold if the environmental temperature drops below 24°C. I should like to state in this connection that in the tropical regions, especially in the West African tropical rain forest region, the outside temperatures show only very slight fluctuations during the day as well as during the year; they vary only between about 24° and 28°C. Normally, therefore, a bat never gets into environmental temperatures which lie below 24°C, and this explains the fact that this temperature stage represents exactly the critical point at which one can go below the activity range of the body temperature with ensuing hypothermic day-sleep lethargy. Beyond this a day-sleep lethargy could be observed also as a normal phenomenon, hence one occurring under natural conditions in a few species, for example, *Rhinolophus landeri;* this observation was made, in fact, on the cool heights of the Cameroon mountain range, where the external temperatures show a greater variation and a much deeper drop than in the purely tropical lowlands.

Just as their representatives in the temperate zones, the tropical bats also awaken of their own accord in the evening from their day-sleep lethargy. They do this, in fact, under the influence of the firmly impressed day rhythm. Likewise, they awaken when they are taken from the refrigerator back into a warm environment. The capacity to regain heat was paralyzed, however, in animals which had remained without food and had not stored sufficient reserve nutrients in their bodies, so that a rise of the body temperature up to the activity range could no longer take place. This strong dependence of metabolism and of heat production on the food intake and the state of nutrition probably, in general, plays a certain role among Chiroptera.

In the small tropical species, *Eptesicus tenuipinnis*, I was further able to show that the day rhythm disappears and no awakening occurs in the evening if the environmental temperature drops below 20°C (Eisentraut, 1940). The day-sleep lethargy then becomes a permanent lethargy, which merely resembles externally the hibernation lethargy of the bats in the temperate zones. In respect to the lethal temperature, it could be observed that in a few tropical species even a protracted stay in an environmental temperature of 8°C or below, with a correspondingly large drop in body temperature, imperils life. These animals then lose the capacity to recover heat and perish, while species of the temperate zones can be cooled without harm below 0°C. This behavior of tropical bats reminds one of Weigmann's observations (1929) on tropical reptiles, among which, likewise, even temperatures above 0°C bring about death through cold.

In summarizing, it can be stated that among Chiroptera, especially among Microchiroptera, quite generally the devices for heat regulation and the capacity to preserve a constant body temperature are very imperfectly developed and have remained at a primitive stage. Therefore, I should like to place the Chiroptera, in respect to their heat economy, on the lowest stage of the lower warm-blooded animals. Kayser (1957) even goes so far as to designate them as poikilothermic.

In connection with the investigation of tropical bats I was interested in the question: How do other tropical mammals behave, whose representatives in the temperate zones are hibernators (Fig. 2, V)? During my last stay in the Cameroons, I had the opportunity to catch a small dormouse, *Graphiurus murinus*, which has its habitat there, and to bring a few living specimens along to Germany. The temperature measurements undertaken on it, determined during a stay in medium environmental temperatures between about 12° and 22°C, indicated a breadth of variation of the activity range from 34.8° to 40.1°C. After transfer into a cold environment (2.5° to 4.5°C) the lower limit of the activity temperature still dropped a bit — in fact, to 33.5°C. But no hypothermic temperatures and no lethargy ensued, as I had actually expected on the basis of a very old statement by Cuvier (according to Heck, 1914). Rather, the animals were able to maintain their body temperature on the level of the activity range. These observations, which are to be continued, have therefore shown up to now that this tropical dormouse also has a great range of variation of the activity tempera-

ture like the European sleeping mouse *Muscardinus avellanarius* (Eisentraut, 1929), which is a hibernator.

At the same time, I had the opportunity to make temperature measurements on a tropical representative of the lorises, *Galago senegalensis*. These measurements have shown that the activity temperature is relatively high and the range of variability, from 36° to 38.6°C, is only relatively small. We can therefore probably include *Galago* among the higher warm-blooded animals, while we must classify the potto, *Perodicticus potto*, previously investigated by me (1956a) and belonging to the same suborder as *Galago*, among the lower warm-blooded animals on the basis of its low activity temperature. Evidently, among the lorises, differing stages in the level of development of the heat economy have been attained. It should be mentioned here that a few Madagascan dwarf-lemurs evidently hibernate, for example, *Microcebus*.

Even the few examples given here show us the considerable differences in the level of development of the homeothermism of the mammals; to a certain degree they give us a picture of the course of phylogenetic development from poikilothermism to homeothermism in a very general way.

On the basis of the results of investigations by Chatfield *et al.* (1948), and by Kayser (1958) on hibernators, the essential difference between the two groups of homeothermic animals seems to me to be the different sensitivity of the central nervous system and of the peripheral nerves to cold stimuli. Lower warm-blooded animals have a broader latitude in this respect and are able, even at lower body temperatures, to maintain their capacity to function and furthermore to endure lower temperatures in the hypothermic state. The lower warm-blooded animals are accordingly distinguished by a physiological or constitutional eurythermism. The same applies also to the hibernators. Physiological eurythermism seems, therefore, to be the prerequisite for hibernation. This is probably also the reason why we find no real hibernators among the higher warm-blooded animals, for instance, among the Carnivora, which must be labeled as physiologically stenothermic.

I have tried to present all these phenomena concerning the heat economy of mammals and in particular of the hibernators only in broad outline and have touched upon many questions only superficially. I am aware that here a broad field of investigation is still open, especially for physiologists. I am also convinced that further investigations of the heat economy in other

species of mammals which have not as yet been considered will open up some new viewpoints or will supplement the present ones.

Summary

Among the mammals we find a very divergent level of development of the heat economy. We can, therefore, distinguish between *higher* and *lower* warm-blooded animals.

In the former, the activity temperature lies above 36°C and its range of variability is small. In the lower warm-blooded animals, the range of activity of the body temperature lies at a lower level and the range of variation is generally greater.

Many phylogenetically old mammals still have an imperfect heat regulation and are lower warm-blooded animals. Among them we must also include the hibernators. The Chiroptera stand on the lowest stage of the homeothermic animals in respect to their heat economy.

Tropical Chiroptera and sleeping mice are compared, in respect to their activity temperatures, with representatives from the temperate zones. Within the suborder of the lorises we find various levels of development of the heat economy.

Lower warm-blooded animals are characterized by a physiological or constitutional eurythermism. Evidently this is the prerequisite for the capacity to hibernate.

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DISCUSSION FOLLOWING EISENTRAUT'S PAPER

ADOLPH asked if the measurement of body temperature was made under controlled (laboratory) conditions or under natural (outdoor) conditions.

EISENTRAUT replied that in most cases the body temperatures were measured obviously under laboratory conditions, but with bats it was occasionally possible to make measurements also in caves under natural conditions.

MORRISON made the observation that it is a wise procedure to make body temperature measurements in the wild, when possible. He felt that hibernators may often be inadvertently placed in a thermic group with primitive mammals, such as marsupials, if body temperature measurements are made under laboratory conditions.

III

COMPARATIVE ECOLOGY OF HIBERNATING MAMMALS

By N. I. Kalabukhov
The Institute of Microbiology and Epidemiology
of the South-East of the U.S.S.R.
Saratov, U.S.S.R.

Introduction

The peculiarities of hibernating mammals have been studied for such a long period of time that our knowledge of this remarkable phenomenon might be considered quite ample.

Comparing the rather scanty ideas on hibernation prevalent at the end of the nineteenth century (see Skorichenko, 1891; Dubois, 1896) with our present diverse and profound understanding of this phenomenon (see Kalabukhov, 1946, 1956a,b; Eisentraut, 1953, 1956; Kayser, 1953, 1957; Lyman and Chatfield, 1955; Herter, 1956) we have to admit that the various aspects of the remarkable deep torpor in animals, which are homeotherms in the active state, have been studied thoroughly.

But in these studies of hibernation in mammals the significance of physiological and ecological peculiarities of mammals in the active state has been undervalued. It is quite obvious that the capacity for going into hibernation for a long period of time should be closely related to certain important characteristics of the mammals observed in their active state, when their bodies are being prepared for the extended period of torpor. Having studied for three decades the phenomenon of hibernation in mammals (Kalabukhov, 1926, 1929a, 1933a, 1935, 1936, 1946, 1956a,b, 1959) we also paid great attention to peculiarities of life of hibernating mammals in the active state (Kalabukhov, 1929b, 1933b, 1938, 1939a,b, 1940, 1954, 1955; Kalabukhov and Raevsky, 1934, 1935, 1936), and we should like to elucidate here some facts related to the problem.

We and our collaborators have especially investigated changes in various species of hibernating and non-hibernating rodents at different seasons of the year. We found it possible to approach closely the problem of ecological and physiological properties of hibernating mammals in the active state, as these seasonal changes were studied not only in different species of hibernating rodents: ground squirrels (Kalabukhov, 1929b, 1938,

1939a,b, 1940, 1950, 1954, 1955; Gerassimenko, 1950; Movchan, 1953; Kozakevich, 1956, 1959), and jerboas (Kalabukhov *et al.*, 1955; Skvortzov, 1955, 1959a,b, Mikhailov, 1956), but also in non-hibernating species, such as gerbils (Kalabukhov and Priakhin, 1954; Kalabukhov, 1956, 1957a,b, 1959; Kalabukhov *et al.*, 1958; Mokrievich, 1957).

Peculiarities of Active Life of Hibernating Mammals

One of the most important characteristics of hibernating mammals is that the principal activities of life such as feeding, storing fat and other reserves, moulting, breeding and growth, all take place in a relatively short period. It is only during the few spring and summer months of active life that hibernators undergo all the changes which enable them to spend an extended period in a state of deep torpor. This capability of accomplishing during a short period of time all the functions which are indispensable for the existence of an individual and species, is a characteristic of hibernators, undoubtedly no less remarkable than their prolonged state of deep torpor. The short spring-summer period of active life causes the hibernators to intensify and accelerate the course of many biological and physiological processes.

Over 30 years ago the American zoologist Shaw (1925, 1926) and Soviet scientists Kashkarov and Lein (1927) called attention to this peculiarity of hibernating rodents represented by ground squirrels *Citellus columbianus* and *C. fulvus* Licht. This observation is confirmed by numerous data showing that the rate of metabolism and the level of chemical thermoregulation in hibernators while in the active state is not lower than in non-hibernators (Gelineo, 1938; Slonim *et al.*, 1940; Slonim, 1945, 1952; Kalabukhov, 1946, 1956; Slonim and Scherbakova, 1949; Sokolov, 1949; Scheglova, 1953).

In Table I we cite data on the rate of metabolism at the critical temperature (25° to 35°C) and its fluctuations when the air temperature is lowered to +10°C in spring (March-April-May) for some species of hibernating and non-hibernating rodents. For this purpose we have taken the data on jerboas, ground squirrels and gerbils. The species of animals are arranged in Table I according to their weight in each group, which enables us to compare mammals of about the same size. Thus we compare three species of strictly night rodents, jerboas (*Alactagulus, Scirtopoda, Dipus*), with three species of gerbils (*Meriones*), which also are active principally at night; and two diurnal species of ground squirrels (*Citellus*), with two rodents also

TABLE I

Oxygen Consumption in Hibernating and Non-hibernating Rodents
(in ml per 1 kilo of body weight in 1 hour)

Hibernators					Non-hibernating Rodents				
Species	Body weight in gms	Consumption of O_2 in ml		Author	Species	Body weight in gms	Consumption of O_2 in ml		Author
		25-30°C	10°C				25-30°C	10°C	
Alactagulus acontion Pall.	22–56	1757–2249	4331–4562	Skvortzov 1959a,b	*Meriones meridianus* Pall.	29–42	2361–2699	4913–5279	Mokrievich, 1957
Scirtopoda telum Licht	39–74	1880–1936	2933–4042	Mikhailov 1956	*Meriones lybicus* Licht	65–87	1350–1591	2597–3519	Kalabukhov, 1956, 1959
Dipus sagitta Pall.	37–90	2162–2264	2296–3373	Skvortzov 1959a,b	*Meriones tamariscinus* Pall.	71–112	1624–1856	2605–2941	Mokrievich, 1957
Citellus pygmaeus Pall.	123–239	1411–1645	2470–2785	Kozakevich 1956, 1959	*Rhombomys opimus* Licht	91–129	1087–1282	2005–2578	Kalabukhov, 1956, 1959
Citellus fulvus Licht	542–873	583–836	1013–1182	Kozakevich 1956, 1959	*Spermophilopsis leptodactylus* Licht	345–550	772–1383	569–807	Kalabukhov, *et al.*, 1958

TABLE II

Seasonal Changes in Resistance to Cooling in *Citellus pygmaeus* and *C. fulvus*

Species	Locality	Period	Changes in the body temperature kept for 1 hour at the temperature				Author
			+5°	+10°	+15°	+25°	
Citellus pygmaeus Pall.	North Ukraine	7-10 April	−3.0	no data			Kalabukhov (1956)
		12-30 June	−5.5°	−2.4°	−1.8°	0.8°	
		5-11 July	−7.7°	−2.3°	−2.0°	−0.8°	
Citellus pygmaeus Pall.	West Kazakstan	6-14 April	−2.9°	−0.7°	−0.3°	0°	Kozakevich (1956)
		10-19 May	−3.7°	−1.8°	−0.6°	−0.5°	
		11-19 June	−5.3°	−3.3°	−1.1°	+0.4°	
Citellus fulvus Licht	West Kazakstan	6-14 April	−2.8°	−1.1°	+0.2°	+0.7°	Kozakevich (1956)
		10-19 May	−3.2°	−2.4°	−1.2°	+0.2°	
		11-19 June	−5.9°	−4.7°	−2.7°	−0.9°	

active in day hours — large gerbils (*Rhombomys*) and long-toed ground squirrels (*Spermophilopsis*). The level of oxygen consumption is higher in almost all hibernators, with the exception of *Alactagulus acontion*, than in non-hibernators of about the same size, both at the critical temperature and at 10°C. The same difference can be observed in small and yellow ground squirrels (*C. pygmaeus* and *C. fulvus*, respectively), both typical hibernators, though they are somewhat larger than the large gerbil and longtoed ground squirrel.

The high rate of physiological processes in hibernators may be largely accounted for by the relative stability of their thermoregulation which enables them to remain active even at a considerable drop of the surrounding temperature (Table II).

The ability to withstand cooling in the hours of their activity, i.e. in daytime for ground squirrels and at night for jerboas, hamsters, hedgehogs and bats, is closely related to the regulative effect of the central nervous system. Slonim (1945), Ponugaeva and Slonim (1949) and Folk *et al.* (1958) reported that bats and ground squirrels in hibernation keep up the rhythm of their activity, while in the spring-summer period a sharp rise in the rate of all processes can be observed at night in bats (Eisentraut, 1934, 1953, 1956). This adaptation is very important, for the activity enables the animals consuming much food to accumulate stores of fat and other reserves indispensable for surviving the state of continuous torpor.

A rapid increase in body weight on awakening after hibernation was observed in hedgehogs (Camus and Gley, 1901) and in captive ground squirrels (Kalabukhov, 1926; Kayser, 1957). A similar rapid accumulation of fat in ground squirrels and marmots under natural conditions was observed by Kalabukhov and Raevsky (1934), Semenov *et al.* (1934), Dubinin and Leshkovich (1945) and Bibikov and Jirnova (1956) (see Fig. 1).

The accumulation of fat at this period is accelerated by a rise in the air temperature which in turn causes a decrease of heat loss. In its turn, the fat stored under the skin and in body cavities decreases the loss of heat and, being a specific factor, inhibits the activity of the thyroid gland (Kratinov and Shkirina, 1947; Leites, 1954), and thus lowers the rate of metabolism. The seasonal changes of the thyroid and adrenals — whose role in regulating metabolic rate in animals is important — have attracted the attention of investigators for a long time (Adler, 1920; Suomalainen, 1938, 1940; Sokolova, 1940; Emme, 1946;

Liapin, 1949; Eisentraut, 1953, 1956; Kayser, 1953, 1957; Uuspää and Suomalainen, 1954; Suomalainen and Uuspää, 1958). However, these changes were studied regardless of the influence of environment.

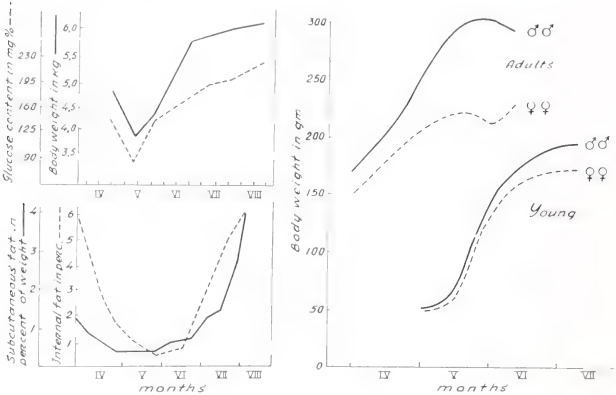

Fig. 1. Changes of body weight in woodchucks and ground squirrels during the period of active life. *Left:* woodchucks, *Marmota baibacina* Kastsh.; *Top:* body weight (——) and glucose content in blood (- - -) (after N. Trukhachev); *Bottom:* percent of subcutaneous (——) and internal (- - -) fat (after Bibikov and Jirnova, 1956). *Right:* body weight of small ground squirrels, *Citellus pygmaeus* Pall. (after I. Mamontov).

Figure 2 illustrates the changes in rate of oxygen consumption in the ground squirrel and jerboa (according to Kozakevich, 1956, 1959; Mikhailov, 1956), showing these metabolic changes in the animal preparing for hibernation. Table III and Figure 3 illustrate gradual changes in the activity of the ground squirrels both in captivity (Kalabukhov, 1939b) and under natural conditions (Kozakevich, 1956, 1959).

That rodents spend less time on the ground surface, diurnal species particularly, is connected probably with the well known influence of shortened day-length on the endocrine glands and on the hypophysis and thyroid in particular (Bissonnette, 1935, 1942; Athonskaya, 1943, 1949; Beliaev, 1950).

Males go into hibernation long before females. The latter store fat more slowly as they have to bear and nourish the young (Kalabukhov and Raevsky, 1934, 1936; Kalabukhov, 1956).

TABLE III

Seasonal Change of Duration of Diurnal Activity in Ground Squirrels in Captivity
(Kalabukhov, 1939b)

Species No.	*Citellus suslicus* Guld Activity in minutes in 24 hours			Species No.	*Citellus pygmaeus* Pall Activity in minutes in 24 hours		
	24/V-18/VI-	16-31/VIII-	Changes in per cent		24/V-29/VI-	11/VII-8/IX-	Changes in per cent
75	120	45	37.8	90	155	78	50.3
77	139	98	70.5	86	169	53	31.4
61	158	94	59.8	39	176	63	35.9
64	176	90	51.1	33	181	137	76.3
73	240	96	40.0	85	225	230	102.2
74	241	39	15.7	88	229	110	47.7
62	245	140	57.1	81	294	78	25.3
65	249	12	24.8	35	299	218	73.0
78	252	197	78.9	87	385	259	67.5
70	259	158	61.6	89	406	159	39.1
60	270	64	23.7				
63	278	169	60.8				
66	331	132	39.8				
69	334	214	64.0				
71	351	123	34.9				
79	371	272	73.2				
Mean	251	125	49.9	Mean	252	139	55.0

NOS. 35, 39, 60, 66, 69, 70, 73, 77, 81 = males, others = females.

TABLE IV

Susceptibility to Plague Infection in the Adult *Citellus pygmaeus* Pall. Before Entering into Hibernation
(Tinker and Kalabukhov, 1934)

Weight in gms.	The dose of microbes in thousands	Duration of life in days	Isolation of the specific culture
		A. Females	
137	50	Over 18(x)	—
107	150	3	+
166	450	Over 18	—
122	1,350	7	+
189	4,050	3	+
101	12,150	5	+
149	36,450	Over 18	—
104	109,050	4	+
		B. Males	
123	50	Over 18	—
120	150	" 18	—
172	450	" 18	—
175	1,350	16	+
122	4,050	Over 18	—
182	12,150	5	+
184	36,450	Over 18	+
175	109,050	" 18	—

(x) "over 18" = The rodents were killed on the 18th day after infection.

TABLE V

Temperature At Which the Mammals Go Into Hibernation
(According to Eisentraut, 1953)

1.	Hamster	9°-10°
2.	Marmot	10°-11°
3.	Hedgehog	15°-17°
4.	Haselmouse	15°-16°
5.	Dormouse	18°
6.	European ground squirrel	20°
7.	Yellow ground squirrel	22°
8.	Tropical bat	24°-25°
9.	European bat	28°

Still later, the young generation (born the current year) goes into hibernation; growing rapidly, they accumulate fat more slowly than the adult rodents. Dubinin and Leshkovich (1945), studying Siberian marmots (*Marmota sibirica* Radde) in the Transbaikal region, stated that some rodents which had insufficient fat before going into hibernation due to poor feeding conditions, either went into hibernation very late, or awakened before the snow melted, and perished.

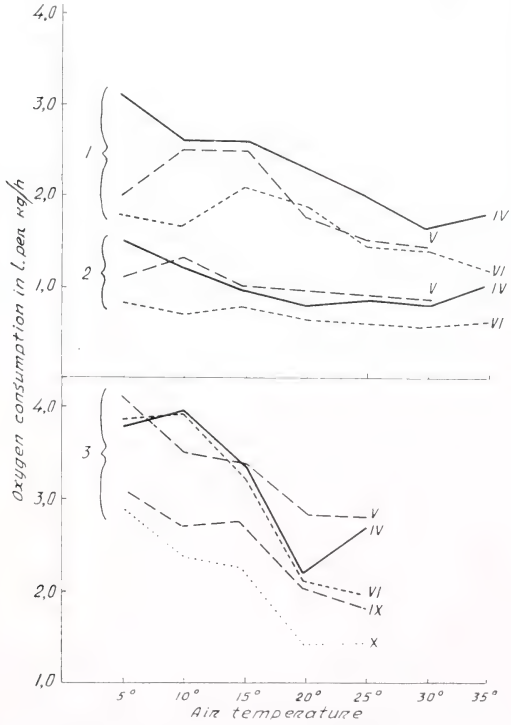

Fig. 2. Changes in the level of oxygen consumption during the active life of hibernating mammals in small (1) and in yellow (2) ground squirrels, *Citellus pygmaeus* Pall. and *C. fulvus* Licht (after Kosakevich, 1956, 1959), and in jerboas (3), *Scirtopoda telum* Licht (after Mikhailov, 1956). Roman numerals = months.

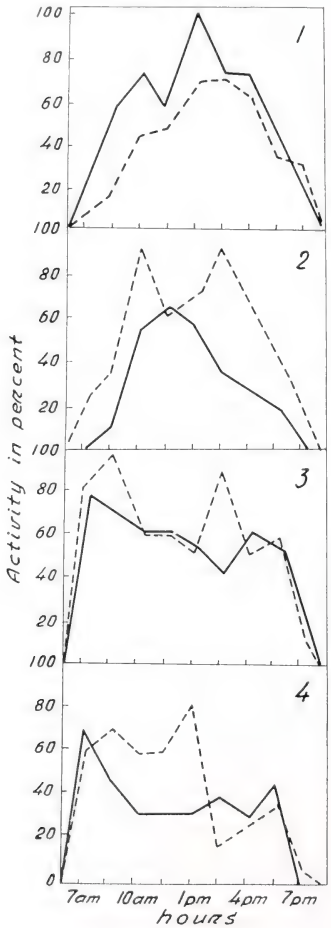

Fig. 3. Changes in the diurnal activity cycle in small (- - -) and yellow (——) ground squirrels, *Citellus pygmaeus* Pall. and *C. fulvus* Licht, in different periods: 1) after awakening; 2) beginning of pregnancy; 3) dispersal of young rodents; 4) before entering into hibernation (after Kosakevich, 1956, 1959).

At the time when fat is being accumulated in the spring-summer period of the life cycle of animals, other no less important components also influence the physiology of hibernators. Kratinov *et al.* (1947) established that during this period ascorbic acid is being stored in the organism of *Citellus pygmaeus* (Fig. 4), and Isaakian and Felberbaum (1949) found the amount of vitamin C to be in direct relation to the condition of nourishment of *Citellus fulvus* in the beginning of summer. Suomalainen (1938, 1940) has observed similar changes in

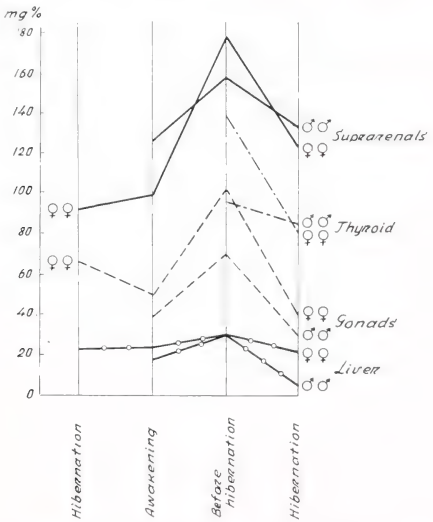

Fig. 4. Changes in ascorbic acid content in different organs of small ground squirrels, *Citellus pygmaeus* Pall. (after Kratinov *et al.*, 1947).

hedgehogs, and has suggested that accumulation of ascorbic acid as an active anti-oxidant inhibits the oxidation of adrenaline in the suprarenal glands, and in this way decreases the intensity of physiological processes.

Then, as demonstrated by M. G. Friedmann (see Kalabukhov, 1956), fat-storing in hibernators appears to be accompanied by storing of fat-soluble vitamin E, which plays an important role, not only in breeding of animals (see Fig. 5), but also in regulating metabolism and heat production as well (Blaxter *et al.*, 1952). It is possible that while the reserves of vitamin E are being stored in tissues, a disconnection of cellular respiration

and phosphorylation takes place and is responsible for inhibition of thermoregulation in mammals (Nason *et al.*, 1957).

Hibernating mammals breed, as a rule, only once a year. Figure 6 illustrates the relationship of the time of breeding for *Citellus pygmaeus* Pall. in different years to the time of their awakening (Kalabukhov, 1929b, 1936, 1956). In Figure 7 is given the periodicity of breeding of certain species of jerboas (Fenjuk and Kazantzeva, 1937; Kondrachkin and Edikina, 1957; Mokroussov, 1957).

It is emphasized that, in spite of the idea that all vital processes are inhibited in hibernation, the very short period of active life suggests a paradoxical fact — namely, that in the period of deep

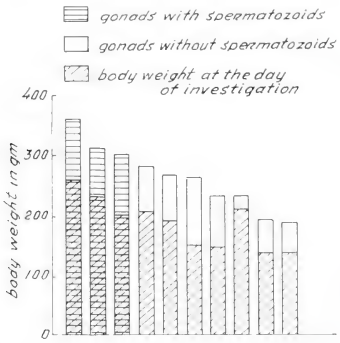

Fig. 5. Influence of the degree of fatness in males of *Citellus pygmaeus* before hibernation (in October) on the preparation for breeding (in March) (after Friedmann; cf. Kalabukhov, 1956).

torpor the body of the hibernator is preparing itself for breeding. This was established for marmots by Rasmussen (1917), and for ground squirrels by Shaw (1926), Sokolova (1940), and Kayser (1953). Asdell (1946), in his book, cites other examples for hedgehogs, bats and rodents.

The peculiarity of the breeding process in hibernating mammals can be explained by the phenomenon of storing vitamin E together with fat. This peculiarity seems to be of great importance as another regulator of the cyclic alterations in the gonads, for changes in the duration of daylight have no effect on hibernators (Allanson and Deansley, 1934; Wells, 1935).

The exclusion of light as a stimulus for the chain reaction from eye to central nervous system to hypophysis to gonads may be

explained by the fact that hibernators stay for months, while in torpor, in deep, dark holes and other covers.

Besides Friedmann's experiments which demonstrated the relationship of the degree of fatness in small ground squirrels before hibernation and the store of vitamin E to their capability of breeding in spring (see Fig. 5), we can refer to the observations of these rodents under natural conditions by Orlova (1955a,b). She reports that when ground squirrels live in summer near wheat fields, they breed next spring more intensively

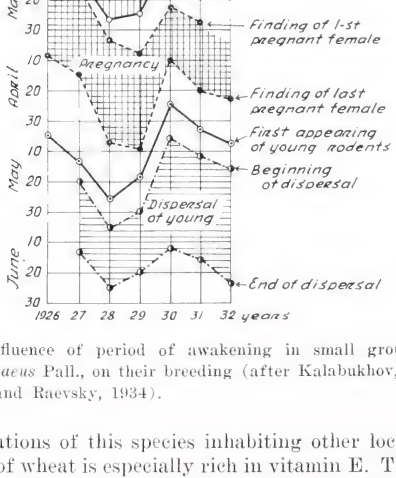

Fig. 6. Influence of period of awakening in small ground squirrels, *Citellus pygmaeus* Pall., on their breeding (after Kalabukhov, 1929b, 1936; Kalabukhov and Raevsky, 1934).

than populations of this species inhabiting other localities. The endosperm of wheat is especially rich in vitamin E. Thus it seems quite possible that going into aestivation in some regions and a retardation of hibernation in others is caused, not only by the drying up of vegetation, but also by different rates of accumulation of fat and other reserves, which in turn depend on different kinds of vegetables and other foodstuffs.

Last of all, the peculiar character of molting in the active period of hibernating mammals distinguishes them from the non-hibernating species which are closely related to them (Kuznetzov, 1940; Hansen, 1954; Kalabukhov *et al.*, 1958). This peculiarity is obviously also influenced by the lack of external stimulus

at the time of torpor, for light is the principal regulator of the process of molt in mammals and in birds active throughout the year (Bissonnette, 1935, 1942; Athonskaya, 1943, 1949; Beliaev, 1950; Kalabukhov, 1951).

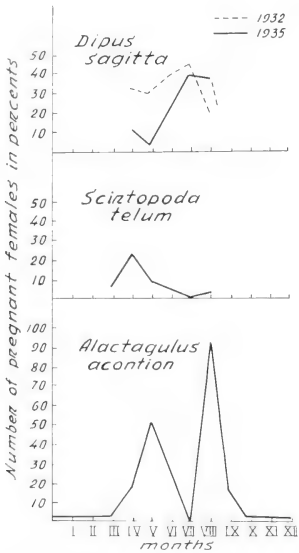

Fig. 7. Cycle of the breeding in different species of jerboas in Precaspian steppes: *Dipus sagitta* Pall. (after Fenjuk and Kazantzeva, 1937), *Scirtopoda telum* Licht (after Mokroussov, 1957) and *Alactagulus acontion* Pall. (after Kondrachkin and Edikina, 1957).

It is evident also that the beginning of torpor in hibernating mammals must be influenced not only by the direct action of external factors on physiological mechanisms but also by the effect of the environment on the behavior of animals. It is an excellent example of the conditioned-reflex relationship in the central nervous system of mammals, or the action on the organism of some external "signals" connected with the approach of unfavorable conditions (Pavlov, 1937). For instance, Ryabov (1948) observed that woodchucks (*Marmota sibirica* Radde)

were active in late autumn, when snow had not yet covered the ground surface, although the air temperature was below 0°C, but went into hibernation on the day after the first night snowfall.

Although desert jerboas (*Dipus sagitta* Pall.) do not usually hibernate in Turkmenia (Vinogradov and Argiropulo, 1938; Kalabukhov *et al.*, 1958; Skvortzov, 1959a), the last author found that they did hibernate after an occasional winter rainfall which caused a dense crust of frozen sand in the region of their dens. Similar lowering of activity of animals under the influence of some external factors is connected evidently with the falling of the level of metabolism and body temperature in hibernators, under conditions of forced, prolonged rest and fasting (Rall, 1932; Isaakian and Felberbaum, 1949; Strumwasser, 1959).

About a decade ago the Soviet investigators discussed the significance of such "signal" changes in the environment, which influence the nervous system of hibernating mammals (Kalabukhov, 1948; Byikov and Slonim, 1949). Now, in the light of modern data about "stress reactions," other scientists have developed ideas concerning the inhibitory effect of external factors on the nervous and endocrine system when hibernators go into torpor (Suomalainen and Herlevi, 1951; Eisentraut, 1956; Kayser, 1957; Strumwasser, 1959). These data confirm our statement that the principal features which characterize the hibernating mammals are closely related to the peculiarity of the active phase of their annual cycle of life.

There are other numerous data illustrating the profound and gradual changes occurring in the organism of hibernating mammals in their active period. Their sensitivity to infection from plague, for instance, is greatly changed. This change in sensitivity is not uniform in males, females and the young, as the rate of physiological modification in their respective bodies is different (Gaisky, 1926; Tinker and Kalabukhov, 1934; Kozakevitch, 1956).

In Table IV these data are given for the small ground squirrel, according to Tinker and Kalabukhov (1934).

According to Kozakevitch (1956) the LD_{50} of plague baeilli increases 37 times for the males of small ground squirrels from the moment of their awakening (March) to the period of preparation for hibernation (June) and only twice for the females. Some fluctuations in the sensitivity of hibernating mammals to various poisons have been recorded at the time of their active life (Borodina, 1956; Kalabukhov, 1956).

The influence of carcinogenic substances, too, seems not to be uniform at different periods of their active life, though this question has been studied as yet only on the mammals in state of torpor (Finkelstein and Rukhov, 1950; Lyman and Fawcett, 1954). Some data on this question is found in a paper by Finkelstein and Belogrudova (1957), who observed the influence of 9,10 dimethyl-1,2 benzantracen on ground squirrels (*Citellus erythrogenus* Br.) in a state of aestivation produced by feeding on dry oats at a room temperature of 14-15°C. Fluctuations in body temperature of 28 torpid rodents ranged between 13.5° and 33.5°C, and between 31.7° and 38°C in 37 active ones. By the end of hibernation neoplasms at the site of injection were considerably fewer in the slightly torpid ground squirrels, than in controls.

All the above-mentioned data and conclusions are of great assistance in another respect, as they help to elucidate a difference having a practical importance, i.e. comparison of the state of hypothermia in non-hibernating animals and in man, to the state of natural hibernation. These phenomena are doubtless not the same. There is little in common between the torpor of hibernators produced by profound and gradual changes in the physiology of the animals in the period of active life and the sudden inhibition of the heat-regulating mechanism produced by drugs or by a rapid cooling of non-hibernating mammals.

The data on hibernation clarifies the rather simplified ideas of artificial hibernation prevalent among some biologists and medical men (Giaja, 1953; Laborit and Huguenard, 1954; Kayser, 1955; Saakov, 1957; Starkov, 1957; Smith, 1958).

All the physiological changes which occur in the active period of the life of mammals that hibernate take a reverse course during hibernation. By the end of hibernation the animals restore their ability to maintain body temperature through a high metabolic rate and other physiological processes, although the surrounding temperature is even lower than at the time when they entered into hibernation (Shaw, 1925, 1926; Kashkarov and Lein, 1927; Kalabukhov, 1929a). This appears to be due to the gradual freeing of the organism from the factors which inhibit the rate of metabolism, e.g. from fat and vitamin E accumulated before going into hibernation which results in restoration of the function of the endocrine glands, especially the thyroid and suprarenal.

The experiments by Suomalainen and Uuspää (1958) which showed that during the awakening of hedgehogs the lowering of adrenaline content and the rise of the noradrenaline level in the adrenal glands took place, also illustrate this regularity, as well as the valuable experiments by Popovic (1955) on the action of desiccated thyroid and methylthiouracil on ground squirrels.

Peculiarities of the Influence of Cooling in Different Species of Hibernating Mammals

That the ability to hibernate appeared in mammals as a result of a definite combination of effects of various external conditions just at the time of their active life can be most clearly confirmed by the fact that the influence of the surrounding temperature on them is not uniform. Eisentraut (1953, 1956) not only divided the hibernating animals into two groups according to the length of their respective periods of hibernation, but he recorded the different degree of their dependence on cooling as well (see Table V).

These data alone are sufficient to disprove the widespread opinion that entering into hibernation is always a result of a drop in the surrounding temperature. However, within one systematic group of mammals such as ground squirrels, jerboas, or hamsters one can find species which become torpid in the high temperatures of early summer, others which become torpid in the low temperatures of late fall, while others do not hibernate at all. Thus, two species, the yellow ground squirrel (*Citellus fulvus* Licht) and the small ground squirrel (*C. pygmaeus* Pall.) go into aestivation in the arid regions of the South-East of the USSR at the end of May and the beginning of June, when the temperature in their burrows is above 15°-18°C (Kashkarov and Lein, 1927; Kalabukhov, 1929a), while in other localities these rodents and a third species of ground squirrel, *Citellus suslica* Guld., go into hibernation only in September when the effect of the low temperature is quite obvious (Tikhvinsky and Sosnina, 1939; Kalabukhov, 1950, 1956).

Still more striking is the difference in the time when two species of jerboas inhabiting the same place — *Scirtopoda telum* Licht and *Alactagulus acontion* Pall. go into hibernation in the Precaspian steppes (Kalabukhov *et al.*, 1955). While the former species become fat as early as July and den up by the end of

October, the latter remain active in November, and sometimes even in December. Thus the double breeding season that was recorded by Kondrachkin and Edikina (1957) in *Alactagulus acontion* Pall. (which distinguished them from the "long-sleeper" *Scirtopoda telum,* studied in the same region by Mokroussov in 1957) does not appear accidental (see Fig. 7). Skvortzov (1955, 1959a,b) established in his experiments that *Alactagulus* goes into torpor only when the surrounding temperature drops below +5° to 6°C, while according to Mikhailov (1956) hibernation in *Scirtopoda* occurs at a higher temperature.

The third species of jerboa, *Dipus sagitta* Pall., is reported to enter into hibernation in the Volga-Ural sands at the same time as *Alactagulus* (Fenjuk and Kazantzeva, 1937; Skvortzov, 1959b), while in Turkmenia it usually remains active throughout most of the winter, although *Alactagulus* becomes torpid there (Vinogradov and Argiropulo, 1938; Skvortzov, 1955, 1959a). It is characteristic of the latter that in the Caspian steppes as well as in the Turkmenian deserts it builds special "hibernating chambers" before becoming torpid (Skvortzov, 1955; Kondrachkin and Edikina, 1957). These chambers have no nest and are situated near the ground surface where the small animal would be easily cooled.

The very peculiar picture of the close correlation between the level of metabolism and thermoregulation and the surrounding temperature has been found in the smallest of hibernating rodents, such as the birchmouse, *Sicista*, or pocket-mouse, *Perognathus* (Suomalainen, 1947; Bartholomew and Cade, 1957).

The greatest variety of reactions to cooling can be observed in the different species of hamsters, among which only the common hamster (*Cricetus cricetus* L.) is a typical "long sleeping" species (Eisentraut, 1928, 1953, 1956) which easily becomes torpid when cooled. Hamsters of the genus *Mesocricetus* such as the golden (*M. auratus* Waterhouse) and the Caucasian (*M. raddei* Nehr.) are less sensitive to cooling (Farrand *et al.*, 1956; Panuska and Wade, 1958). It was in the golden hamster that South (1958) discovered that the rate of oxygen consumption in the cardiac muscle was lower than in the bat, and thus the "heat of activation" is higher in the golden hamster than in true hibernators such as the bat.

Finally, many species of hamsters (genus *Cricetelus*) which inhabit the steppes and desert regions of Europe and Asia (*Cricetelus migratorius* Pall., *C. eversmanni* Brandt, *C. bara-*

bensis Pall., *Phodopus sungorus* Pall.) fail to hibernate, and stay active throughout the year (Kalabukhov, 1956).

In a state of torpor the sensitiveness to cooling in different species of hibernating mammals is also not uniform. Some of them awaken when the surrounding temperature drops to 0°C or below which suggests quite a paradoxical ability of restoring heat regulation on cooling as well as on warming. This was recorded in the ground squirrel by Horvath (1881), and in the hedgehog by Suomalainen and Suvanto (1953), and Chao (1955). On the contrary, other species can stand cooling to a temperature below 0° for a long time, the liquids in their body being in the state of supercooling at –1.0° to –1.5°C in small and long-tailed ground squirrels, or even at –5° to –9°C in bats (Bakhmetiev, 1912; Kalabukhov, 1933a, 1935, 1958; Murigin, 1937, 1948; Nekipelov and Peshkov, 1958).

Conclusion

All the above data permit us now (Eisentraut, 1953, 1956; Kalabukhov, 1936, 1946, 1956; Lyman and Chatfield, 1955) to reject the doubtful idea that the ability of some species of mammals to hibernate is but a manifestation of the primitive characters of poikilothermal animals.

We believe that the above data show that the phenomenon of hibernation is not only a result of adaptation in animals to the unfavorable conditions of life in autumn and winter but also to consequent extreme shortening of the duration of the phase of their active life.

Without considering many other facts mentioned in our book (Kalabukhov, 1956b), we wish to complete our paper calling to mind the statement by Charles Darwin (1839), in the fifth chapter of the "Journal of the Voyage of the H. M. S. Beagle," on the significance of the temperature factor in hibernation: "This shows how nicely the stimulus required to arouse hibernating animals is governed by the usual climate of the district, and not by absolute heat." If his idea included also entering into hibernation, we find an excellent example of how profoundly the great scientist understood the degree of relativity in all adaptations to the conditions of existence.

I am sure that recalling these wise words is particularly appropriate in the current year, since the 150th anniversary of Darwin's birthday occurred in February, and in a few months the centennial of his "Origin of Species" will be celebrated.

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IV

SOME INTERRELATIONS BETWEEN WEIGHT AND HIBERNATION FUNCTION[1]

By Peter Morrison
Departments of Zoology and of Physiology
University of Wisconsin
Madison, Wisconsin

The phenomenon of hibernation in mammals which has attracted the interest of zoologists for more than 200 years, provides the first demonstration in comparative physiology of a striking adaptation to special environmental requirements, and at the same time emphasizes the contrasting homeostatic conditions in other mammals. The general conditions of hibernation were laid out in several studies early in the last century, and these showed the depressed level of metabolic activity as manifested by a low body temperature and reduced heart and respiratory rates and suspension of all general activities. Since that time a considerable body of data has been collected and with the improvement of physiological technique and instrumentation we now have detailed descriptions of many aspects of this condition. These data have shown common features in many animals but also substantial quantitative differences between species in such factors as the duration of hibernation, the accumulation of metabolic reserves, the reduction in body temperature (T_B), and its relation to the ambient temperatures (T_A), the degree of depression of metabolic activities, and the specialization of individual tissues for functioning at low temperatures. Thus, the jumping mouse, the hamster, the hedgehog, the ground squirrel, the bat and the bear may each be distinguished from the others in respect to at least one feature of its hibernating behavior. As a consequence, there is some difference of opinion as to the precise definition and limits of the condition of hibernation, and as to the animals which should be classed as hibernators. Thus, how greatly must the metabolic level be reduced for an animal to qualify as a hibernator? Must the body temperature be lowered by a specified amount, or must it approach the ambient temperature within a specified limit?

[1] Studies on hibernation at the University of Wisconsin have received continuing support from the Wisconsin Alumni Research Foundation.

The examination, which follows, of some underlying principles related to energy balance and hibernation will not directly answer these questions, but it may provide some basis for a common treatment of various animals, and clarify the relation of questionable species both to acknowledged hibernators and to the bulk of non-hibernating mammals.

All animals may encounter periods during which food is unavailable or in short supply so that a deficit must be met from body reserves. During hibernation the ordinary metabolic demands, which might otherwise exhaust the metabolic reserves, are reduced to such a level that the reserves last through a whole inclement season. Although protein is depleted during prolonged starvation, this represents a loss of structural material, and the metabolic reserve may be considered as effectively represented by the body fat. The fat content[2] may vary widely, but its maximum level (F_M) may be taken as about equal to the fat-free[2] body weight (W).

$$F_M = W(\text{g}) \qquad (1)$$

Although wild animals ordinarily carry much smaller amounts of fat (G. C. Pitts, personal communication), such values, i.e., 50 per cent of *total* body weight as fat, are observed in species spanning almost the entire weight range from the jumping mouse (10^1g) to the blue whale (10^8g), and there are no obvious intrinsic limitations which would prevent such an accumulation in any animal.[3] Thus the potential energy reserve will be proportional to body weight in animals of all sizes and may be expressed as

$$F_M = 7W \text{ (kcal) (W in g)} \qquad (2)$$

By contrast, the basal metabolic rate (M_B) is weight dependent, following the familiar relation,

$$M = 0.44^{3/4} \text{ (kcal day}^{-1}\text{) (W in g)} \qquad (3)$$

(exponent rounded; Brody, 1945). Therefore, the fasting potential will vary directly with weight and represent the quotient of these two heterogenic relations (Adolph, 1949) in animals with a "maximum" fat supply and a "standard" metabolic level.

$$F_M/M_B = 16\ W^{1/4} \text{ (days)} \qquad (4)$$

Or the fasting duration can be more generally described as

$$F/M = f/m\ 16\ W^{1/4} \text{ (days)} \qquad (5)$$

where f is the fat content (fraction of W) and m is the metabolic level (fraction or multiple of M_B).

[2] For the purposes of this discussion "fat" will represent adipose tissue, the triglyceride content of which will be taken as 75-80% (Pitts, 1956).

[3] Bats may represent an exception in that the requirements of flight could limit the load of fat. In *Eptesicus*, a maximum fat content of ⅓ ($f = ⅓$) has been observed prior to hibernation (Beer and Richards, 1956).

This relation is shown in Figure 1, which relates log days of fast to log weight. The heavy curve shows the relation when $f = m = 1.0$, and the light curves show the effect of lowered fat content with f ranging down to a value of 1/8. An adequate duration for survival might be taken as 200 days (7 months), the time between first and last frosts in our northern states, although dormant periods as long as 300 days have been described (Volcanezkÿ and Furssajeu, 1934), and shorter periods, of about 100 days, may be adequate under other circumstances. At the M_B, a 20 kg animal (beaver) might just survive 200 days with the maxi-

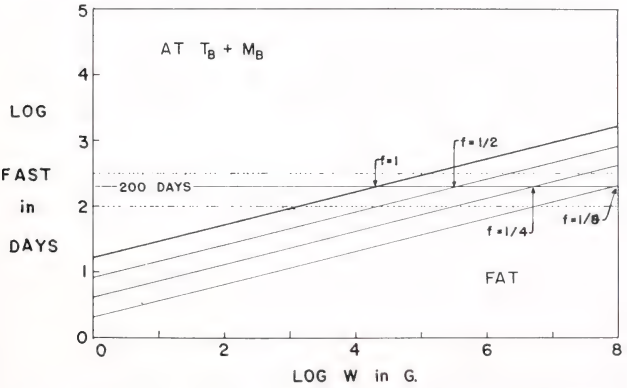


Fig. 1. Influence of body fat on fasting time in mammals of different size ($m = 1$).

mum fat content, but a 300 kg animal (bear) would need only half this amount. A 5-ton animal (elephant) would meet the 200 day requirement with only 1/4 its weight in fat (20 per cent *total* weight), and our largest mammal (blue whale) would last with $f = 1/8$. Animals smaller than 20 kg could not survive without modifying their metabolic level. It may be noted that our largest unquestioned hibernator, the marmot, has a weight just below this value. A 200 g animal (squirrel) could survive about 2 months, and our smallest mammal (3 g shrew) only 20 days even with 50 per cent of the total body weight as fat.[4]

[4] No hibernating shrews are known, and how small shrews meet the substantial requirement of several times their body weight in meat each day during the winter is a question. However, it is amusing to consider that the long-tail shrew might have a vicarious connection to hibernation. In Alaska these shrews may be found in areas with Arctic ground squirrels, and should the latter be available, one large hibernating carcass could support half a dozen 3 g shrews over the entire winter. Reports of shrews scavenging animals as large as a moose suggest that this possibility is not as fanciful as it may seem.

Figure 2 shows the effect of a reduced metabolic level on survival ($f = 1$). It may be seen that with a factor, $m = 1/2$, a 2 kg animal (marmot) will survive for 200 days; at $m = 1/4$, a 100 g animal (ground squirrel); and at $m = 1/8$, a 10 g animal (small bat). However, in interpreting these curves it will be judicious to allow some margin, perhaps 2-fold, for increases in metabolism either during the periodic awakenings which appear necessary or at the end of the fasting period in the spring. For example, the metabolic rate in naturally hibernating *Myotis*, estimated

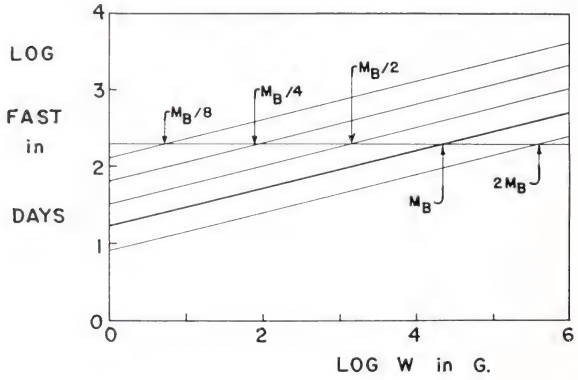


Fig. 2. Influence of metabolic level on fasting time in mammals of different size ($f = 1$).

from fat depletion, is 0.1 cc 0_2/g/hr (Mann, 1936) while the measured metabolism at 5° C is 0.05 cc/g/hr (Hock, 1951). Similarly, oxygen consumed during periodic awakenings in ground squirrels and hedgehogs is of the same magnitude as that used in the intervening dormant periods.

It may also be more appropriate to estimate fat on the basis of $f = 0.5$, rather than $f = 1.0$. Although the latter value is a reasonable maximum, the former (33 per cent of total weight) may better represent ordinary values.[3] So, for these average values ($f/m = 1/4$).

$$F_A/M_A = 4\ W^{1/4}\ (\text{days}) \qquad (6)$$

With this conservative estimate, a weight of more than a ton is necessary for a 200 day fast.

As the temperature of a hibernating mammal falls, so does its metabolism, and this reduction may be calculated if a temperature coefficient is assumed. Values near 2.0 are characteristic of many biological systems and have been described for isolated tissues in hibernating and other mammals (South, 1958; Meyer and Morrison, 1960).

Figure 3 shows the effect of lowering the temperature in such a system. With a 30° drop, a small bat would last the 200 days, but with no margin for a lower average fat level or a higher average metabolism (cf. above). For $f/m = 1/4$, a kg or more of body weight would be required. A further depression of the

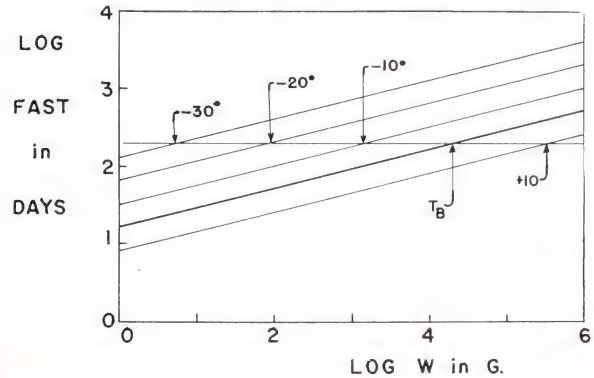


Fig. 3. Influence of body temperature on fasting time in mammals of different size ($Q_{10} = 2.0$ and $f/m = 1$).

metabolism is required in smaller mammals and such is indeed seen (Kayser, 1940), but this necessitates a higher Q_{10}. The influence of variation of Q_{10} on the fast duration at a representative hibernating temperature of 10°C ($T = -25°$) is shown in Figure 4 for various Q_{10} values. At $f/m = 1/4$, a Q_{10} of 4.0 is necessary for survival of a small bat. Interestingly, a Q_{10} close to this, 3.8, may be calculated from the data of Hock (1951) on *Myotis*, between 2 and 40°C.

Another quantity relating to hibernation that may depend on body weight is the difference between the ambient and body temperatures. Figure 5 shows this in an experiment on a thirteen-lined ground squirrel, in which the T_A passively followed the T_B

but always at a slightly higher level by virtue of its heat production. In popular terms such hibernation represents a turning-off of the physiological "thermostat." It may be left off indefinitely, in which case the temperature will always "float" just above the ambient temperature, as in the bat; or, as in the hedgehog or dormouse it may be subsequently "set" at a new level in response to a low T_A, so that the animal again regulates but at a T_B far below that of the active animal (Eisentraut, 1929). Animals just entering hibernation may also show intermediate states (Strumwasser, 1959) or an alteration of "on" and "off"

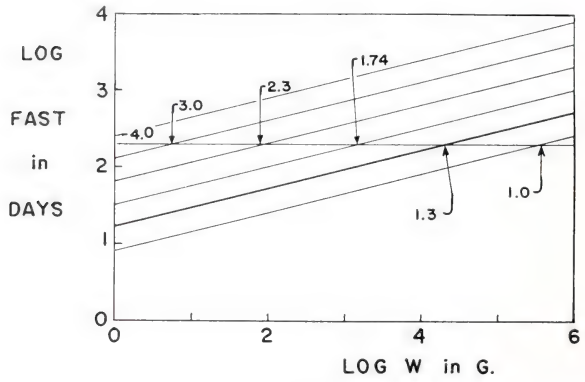


Fig. 4. Influence of Q_{10} ($T_B = 25°$) on fasting time in mammals of different size ($f/m = 1/2$).

cycles, but the passive condition with the T_B close to the T_A should probably be considered the ordinary situation.

These small temperature differences are difficult to measure unless the T_A is carefully controlled. But they may be directly estimated from the metabolic rate (M) under the given conditions and the thermal conductance (C). These quantities are related through Newton's law of cooling as applied to a thermoregulating animal (Scholander *et al.*, 1949). Thus,

$$M = C\ (T_B\text{-}T_A) = C\,\Delta T \qquad (7)$$

$$\text{or } \Delta T = M/C \qquad (8)$$

Metabolic estimates of C (Morrison and Ryser, 1951), supplemented by *in vitro* measurements on pelts (Scholander *et al.*, 1949), give the relation:

$$C = K\ W^{1/2} \tag{9}$$

That is, because of the decreasing surface to weight ratio and the increasing pelt thickness, larger animals cool less rapidly than smaller ones. If we now combine equations (3), (8) and (9),

$$M/C = \Delta T = m\ k\ W^{1/4} \tag{10}$$

and the temperature difference will vary directly with weight. However, the use of this equation for animals in hibernation at any given T_A presumes a common temperature coefficient. If, as Kayser (1960) suggests, the metabolic-weight function during hibernation has an exponent near 1.0, then the temperature difference may be even more weight dependent:

$$\Delta T = k\ W^{1/2} \tag{11}$$

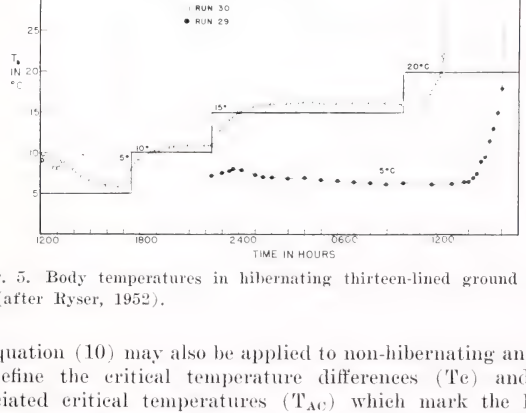


Fig. 5. Body temperatures in hibernating thirteen-lined ground squirrels (after Ryser, 1952).

Equation (10) may also be applied to non-hibernating animals to define the critical temperature differences (Tc) and the associated critical temperatures (T_{AC}) which mark the lower limit of thermal neutrality and the beginning of chemical regulation.[5]

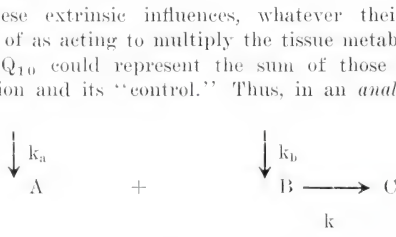
$$M_B/C = T_B\text{-}T_{AC} = \Delta Tc = 4\ W^{1/4} \tag{12}$$

It has, moreover, a possible relation to hibernation, since an animal in the ordinary hibernating posture which allows minimum heat dissipation cannot reduce its body temperature in the zone of

[5] By coincidence, this equation (12) is identical to equation (6). That is to say, the critical temperature differential in °C is equal to the fasting period ($f/m = \frac{1}{4}$) in days.

thermal neutrality without lowering the metabolism below the basal level. While some animals possess this ability to abruptly lower their metabolic level (V. Popovic, personal communication) this capacity may be limited to species which become dormant in warmer latitudes or seasons.[6] In our experience temperate or northern hibernators do not show abrupt metabolic depressions. In one species, the jumping mouse (*Zapus hudsonius*), individuals ready for hibernation were observed to starve to death at 25°, neither eating available food nor lowering their body temperature or metabolism. In *Myotis* (Hock, 1951) or in the Arctic ground squirrel (Fig. 6), the metabolism follows along a single temperature function during the course of cooling. This function ($Q_{10} = 4\text{-}6$) is considerably steeper than can be accounted for by the intrinsic behavior of the isolated tissues (South, 1958; Meyer and Morrison, 1960) and so must involve regulatory components. However, the observed regularity in behavior appears more compatible with a passive response to temperature than to an active depression of the metabolic level at any point.

If these extrinsic influences, whatever their nature, are thought of as acting to multiply the tissue metabolism then the overall Q_{10} could represent the sum of those for the tissue respiration and its "control." Thus, in an *analogue*:



If the concentrations of A and B depend on their rates of formation, k_a and k_b, which are in turn governed by temperature coefficients, Q_{10a} and Q_{10b} then $k = k_a\ k_b$ and $Q_{10} = Q_{10a} + Q_{10b}$. By such a concept we might account for the high observed Q_{10} values as the resultant of two Q_{10} values of ordinary magnitude, which act together to maintain a single overall temperature coefficient over the whole range.

[6] Such a capacity to depress the metabolic level with little reduction in T_B might provide a physiological criterion for differentiating aestivators from hibernators. Since both classes appear to behave in a similar manner during dormancy, this would represent an added specialization in the aestivator.

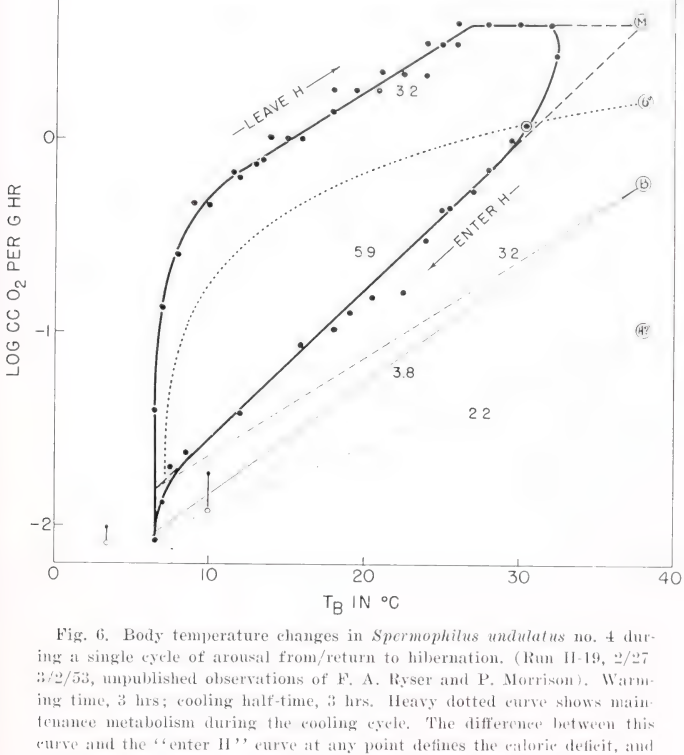


Fig. 6. Body temperature changes in *Spermophilus undulatus* no. 4 during a single cycle of arousal from/return to hibernation. (Run II-19, 2/27 3/2/53, unpublished observations of F. A. Ryser and P. Morrison). Warming time, 3 hrs; cooling half-time, 3 hrs. Heavy dotted curve shows maintenance metabolism during the cooling cycle. The difference between this curve and the "enter H" curve at any point defines the caloric deficit, and corresponds closely to the observed rate of cooling, e.g., 2.7°/hr. @ 20°. "M," maximum observed metabolism in this individual in summer; "6°," maintenance metabolism at $T_A = 6°$ and $T_B = 38°$; "B," basal metabolic rate in summer; "⊙," maintained metabolism during 4-hour period. Values refer to Q_{10} for each curve. Note the identity in Q_{10} between "leave H" and light dashed curves which differ only in rate constant (factor of 60). The fine dotted curve was drawn with the Q_{10} of 2.2 seen in isolated tissues. The difference between its value at 38° ("H?") and M_B (factor of 6) may define the "extrinsic" depression of the metabolism in hibernation. The light solid line curve ($Q_{10} = 3.8$) corresponds to the values for the transition into hibernation reported for other hibernators. ⊙ designates early and later readings from other animals in hibernation.

Accordingly, the critical ambient temperature may represent a limiting condition, and only below this value could our (northern) hibernators lower their T_B by failing to supply heat for thermoregulation. Model curves for two animals of different size are given in Figure 7. The 40 g animals (e.g., a jumping mouse) could cool below 25°, but the 4 kg animal (e.g., a large marmot) would need a temperature of less than 5° to cool. Of course, these relations are based on the standard curve of metabolism and weight, in which *Marmota* is a conspicuous exception with a metabolic rate about one-half the "predicted" value (Benedict and Lee, 1938). Thus, the critical temperature for

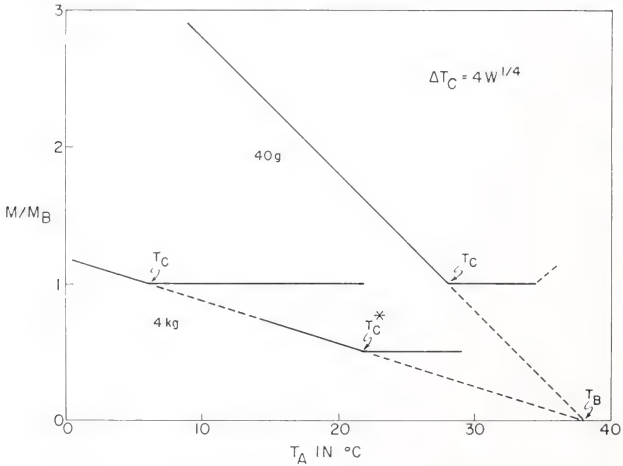


Fig. 7. Model curves for two animals showing the influence of size and basal metabolic rate on the critical temperature, below which the body temperature can be reduced while metabolism remains at the basal level.

the marmot would be 20°C, and not 5° which might be difficult to attain. One wonders if this correlation with hibernation function and considerable size could provide an explanation for the unusually low metabolic rate in this genus.

The relation between conductance and weight is only valid up to a size of perhaps 50 kg since the fur length does not increase in larger animals. At this weight, however, the critical differential would be 65° and the critical temperature −30°C.

It will be of interest to evaluate the bear in terms of the previous discussions. This animal occupies an anomalous position among hibernating mammals. Although it is popularly the animal first thought of in reference to this phenomenon, some naturalists and physiologists suggest that the bear is not a hibernator at all. This view stems from observations of reactivity and body temperature during the dormant phase. These show that while some bears are certainly inactive for a considerable season, they do not become torpid and helpless as do most hibernators, but may react with considerable vigor at any time and can carry out activities, e.g., parturition, which seem incompatible with hibernation. Further, the body temperature clearly does not fall near the ambient temperature, although depression of the T_B below the normal range (to 31°+) is reported from measurements on a denned bear (Hock, 1957). Other values on a captive bear (Svihla and Bowman, 1954) and a recent measurement, from our laboratory, on a denned bear in Wisconsin indicate T_B values within the normal range.

We have just noted that because of its low critical temperature (equation 12) the bear should have difficulty in cooling, and this would be so even with a considerable increase in conductance. Further, it may also be calculated that because of its low thermal conductance (equation 9) it could not maintain a T_B near the T_A. In any event, it is clear (equation 5) that the bears lie in the interfacial zone between animals which might last through the winter at or near their basal metabolic rates and those which cannot. A small bear with little fat would fall in the latter group, while a large bear with abundant fat would definitely fall in the former category. Our Wisconsin bear (cf. above) had more than enough fat to last through the winter without metabolic alteration.

Even in the bears which need some extension of metabolic reserves, the requirements will be much more modest than in smaller animals. Thus, the limited observed depression of T_B and metabolism (Hock, 1957) would be sufficient to extend the fasting period by a critical factor of perhaps 2-fold, and thus allow survival in nature. This may qualify the bear as an "ecological" hibernator. On the other hand, the limited extent of these changes and the lack of adaptations for functioning at low temperatures[7] may disqualify the bear as a "physiological" hibernator.

[7] The properties of peripheral nerve in the bear are similar to the non-hibernating white rat rather than to the hibernating ground squirrel (Kehl and Morrison, 1960, and unpublished observations).

Given the ability to hibernate at all, i.e., to turn off or readjust the "thermostat," one may ask: Why did these species not evolve toward an even more economical hibernation with even lower T_B and metabolism in the winter. This could be very important in some situations, e.g., a poor season with limited fattening. Perhaps, the size relations (equation 10) prevent substantial lowering of the body temperature. However, even were it achieved, this lower T_B would present a distinct concomitant disadvantage to the bear which, because of its size, is often only poorly protected from disturbance. No one who has encountered a denned bear will question the survival value of this intermediate condition which, while permitting a substantial saving of energy by complete inactivity and perhaps some lowering of T_B, at the same time maintains the animal in condition to take immediate defensive action. This is clearly in contrast to smaller hibernators, which, though helpless when disturbed at a low temperature, are in nature effectively protected from such disturbance by niches or burrows. It may be significant that the T_B near 30° in the denned bear is just low enough to provide a reasonable reduction in metabolic rate (factor of 2), and is also just high enough to provide effective activity for defense. At any rate, a temperature near 30° appears critical for effective action by other hibernators such as the bats (Reeder and Cowles, 1951), which on occasion actively maintain their T_B at this level (Morrison, 1959).

The behavior of the bear is, then, indisputably distinct from most hibernators, but the question of whether it hibernates or merely enters a deep sleep will depend on our definitions of these respective states. However, some of the controversy regarding this animal may be resolved in terms of a flexible response to meet different needs and circumstances.

Other medium-sized carnivorous animals including the skunk and badger have been suggested as possible hibernators because of their disappearance in the winter over considerable periods when their normal food supply may be unavailable. But their dens or burrows do not permit direct observation or removal without disturbances, and they have not been induced to hibernate in the laboratory. However, negative evidence may merely indicate inadequate conditions, since captive bears are seldom dormant. Slonim (1952) has described instability of body temperature and metabolism in the badger during the winter and

although deep hibernation was not observed, the general behavior appeared not unlike that of the bear.

The energetic considerations advanced above for the bear are even more cogent as applied to these smaller species. No conceivable amount of fat would tide these animals over a winter season without a very substantial reduction in metabolism. However, their reserves would be sufficient to last them over periods of more than a month or perhaps two months with a modest metabolic reduction of 2-fold as seen in the bear. Disappearances of such duration have been noted during periods of inclement weather.

Animals with adequate food either in stores or accessible in the environment need not fast. Fasting (and hibernation) is only found in animals with inadequate external food reserves, whatever the reason. Animals without external reserves must use internal reserves and often hibernate. However, there are intergrades such as the hamster which may either hibernate or use a storage food. These might be referred to as *permissive* hibernators, which may regularly have an option between hibernating or not during the winter. These contrast strikingly to such a form as the jumping mouse which does not store food, but is so *obligated* to become dormant in winter that, when conditions are not suitable, it starves to death rather than modify its habits or its metabolism.

Although we have confined our attention to mammals, it should be noted that the problem of the extension of food reserves over an inclement season is a common one to most cold-blooded animals as well, and equations (1)-(6) may be modified for their use. But the problem is more critical in homeotherms for several reasons. The first is their higher metabolic rate, which in part reflects their higher temperature. Thus, at 20° the rattlesnake requires only 0.1 cc O_2/g/hr and is therefore comparable to the whale in maintenance efficiency. The second disadvantage in homeotherms is that their winter requirements may increase due to the demands of thermoregulation, while those of poikilotherms are substantially reduced. Finally, the organization in homeotherms may be considered as of a more critical nature with fixed maintenance requirements while in the lower animals a considerable lability is tolerated. Table I summarizes these concepts.

TABLE I

Equivalent Influences to Extend Fasting Duration by Two-fold.

FACTOR	CHANGE
W	x 16
m	x 1/2
f	x 2
T_B	-10° ($Q_{10} = 2.0$)
	-7° ($Q_{10} = 3.0$)
Q_{10}	x 1.42 ($T_B - 20^\circ$)
	x 1.26 ($T_B - 30^\circ$)

In conclusion, we have brought together several simple equations which interrelate body weight, fat content, metabolic rate, ambient and body temperature and thermal conductance. These equations which relate common aspects of the energetic balance in hibernating and non-hibernating animals can be used as a framework on which to present various hibernating species. These equations conveniently show the quantitative substitution of the various factors which both necessitate and permit hibernation. They also show the gradient in the summed requirements for hibernation in animals of increasing size, and, with the decrease of the sum to zero in larger animals, allow some prediction as to the size limit of hibernators. The qualitative influence of the various factors described here are well known, but it is hoped that these relations may be useful in bringing them together and allowing the synthesis of a variety of observations into a common pattern.

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DISCUSSION FOLLOWING MORRISON'S PAPER

STRUMWASSER suggested that it becomes a semantic problem to decide on criteria of hibernation when the quantitative aspects become the all important considerations. Instead, one might define hibernation qualitatively, yet operationally. Broadly speaking, hibernation might be considered the regulation of a set of physiological parameters at preferred levels leading to minimal energy expenditure; obviously there are different degrees of hibernation. The regulation of a set of physiological parameters at some new preferred level is hardly an unknown phenomenon in biology; the principle is evident in animals escaping from predators and might be expected in preparations for migratory flight in birds. STRUMWASSER then asked MORRISON what he meant by "turning off the thermostat." MORRISON replied that to his mind "turning off the thermostat" was the simplest way of envisioning hibernation taking place. He pointed out that adaptations are generally conservative, that if possible the animal modifies what is available rather than evolve a new mechanism. Among bats, for example, there is no question but that the "thermostat" is turned off rather than reset at some lower level and some marsupials seem to have the same ability.

A second question by STRUMWASSER was, "What is the Q_{10} of metabolism during the actual entrance into hibernation?" The reply was that to his (MORRISON's) interpretation, an animal could simply "slide down" a temperature-metabolism

function. In general, he saw no difficulty with this as a response pattern, and concluded that once the "thermostat was turned off" the system could respond passively.

PENGELLEY asked whether a marsupial which "turned down its thermostat" was able to lower its body temperature to levels comparable to hibernation. MORRISON replied that he had recorded marsupial body temperatures of below 30° at ordinary ambient temperatures, but observations at lower ambient temperatures have not been made.

DENYES stated the well-known biochemical doctrine that "fat is burned in the flame of carbohydrate." Thus, if an animal is using fat, it must use carbohydrate to make this possible. In fat loss the limiting factor may be carbohydrate supply and the animal then arouses. If an animal ends the hibernating season with a residue of fat, this strengthens the contention that carbohydrate is the true limiting factor. MORRISON stated that the problem of intermediary metabolic rate and fat utilization in these animals was extremely difficult of solution.

V

TORPIDITY IN BIRDS

By Oliver P. Pearson
Museum of Vertebrate Zoology
University of California
Berkeley, California

Birds and mammals are called homeotherms in spite of the fact that their body temperature changes under varying circumstances. All of them operate near the upper temperature tolerance of their bodies and so all resist rising body temperature with whatever behavioral and physiological tricks they can perform. The response to lowered body temperature, however, is quite different in different species. When considering this response in mammals I have found it convenient to set up three categories: (1) obligate homeotherms; (2) stubborn homeotherms; and (3) indifferent homeotherms.

Obligate homeotherms. The species in this category, when in a cold environment, work with all their resources to retain a high body temperature. When they have exhausted themselves in this effort their temperature drops to a level at which they cannot survive and from which they cannot recover. Man and probably most other mammals fall in this category. These are the chaste mammals, defending their thermal virginity to the death against the onslaughts of the environment. Not all members of this category are as precise regulators as fully clothed man; drops of several degrees centigrade when in cold surroundings are not unusual, as well as slightly lowered temperatures during sleep.

Stubborn homeotherms. These animals maintain a warm body temperature over a wide range of environmental temperatures, but under the influence of excessive cold or of hunger the body temperature drops drastically. The animal can, however, recover from the accompanying torpor and return to normal active life. *Peromyscus* (deer mice) indulge in this emergency torpor in the wild (Howard, 1951), and several species of *Perognathus* (pocket mice) drop into torpor in captivity when food is withheld (Bartholomew and Cade, 1957). These are the submissive creatures whose thermal virginity will yield to appropriate environmental pressures.

Indifferent homeotherms. In this category fall the least precise temperature regulators, such as most species of Temperate Zone bats, in which the body temperature drops almost to that of the surroundings whenever they fall asleep in cool or even in moderately warm surroundings. From this torpor they arouse themselves spontaneously just as other animals awaken from sleep. These are the animals of easy virtue, the promiscuous ones.

True hibernators (those that remain in deep torpor for many days or weeks) can belong either to the second or to the third category. Syrian hamsters in captivity seem to belong to the second category (stubborn homeotherms) because they usually require a little push (exposure to cold) to make them torpid. In addition to bats, various hibernators such as jumping mice (*Zapus*) belong in the third category, because a *Zapus* kept at room temperature with an excess of food and water will spontaneously enter hibernation. Admittedly, the boundaries between the three categories are not sharp. A species or an individual may shift back and forth between the second and third category depending upon the time of year, endocrine balance, etc. Nevertheless, I find the classification useful. Whenever I meet an unfamiliar species, I like to know into which category it falls, for this helps to define its metabolic personality.

Among birds the same three categories are appropriate. Most birds fall into the first category, obligate homeotherms, and maintain a warm, fairly stable body temperature throughout a wide range of environmental temperatures. Most birds are strictly diurnal and probably more lax than mammals about body temperature while they are asleep, but, although many birds permit their body temperature to drop 2°C or more when they sleep (accompanied by a drop of 10 per cent or more in metabolic rate), unlike sleeping bats they continue to regulate their temperature nicely at the lower sleeping temperature. Therefore, the birds in this category, the obligate homeotherms, are of no further interest in this discussion. It is in the other two categories that we find torpor.

Much of the literature on torpidity in birds has been discussed in a valuable review by McAtee (1947). He pointed out that scientists have been slow to acknowledge the occurrence of torpidity in birds, in spite of dozens of seemingly reliable reports. Within the past 15 years, however, torpidity has been demonstrated conclusively by physiological criteria in several species, and it is reasonable to assume that other species will be added to the list in the future. It is certain that we still have much

to learn about the nature of torpor in those species already known to become torpid. I shall review what has been learned so far about torpor in the few species that have received more than minimum attention.

Swifts. Swifts make their living by sweeping through the air in pursuit of flying insects. One of the risks accompanying this way of life is the danger of starvation when bad weather keeps the poikilothermous prey grounded while the warm-blooded predator must keep its machinery running at almost full speed. Swifts and many other birds migrate to warmer lands and thus are not concerned with the over-winter scarcity of flying insects, but this does not solve the problem of reduced food supply during spells of stormy weather in the summer nesting season. Most species of small birds will die in a few days without food, yet swifts live in many regions where several consecutive days of bad weather are not only a possibility but a probability. Apparently in response to this environmental challenge swifts have developed the ability to drop into temporary torpor, thereby slowing until the return of good weather the rate at which they use up the food resources stored in their tissues.

Koskimies (1948, 1950) has done the most pertinent research with swifts. He reports, for example, that nestling European swifts (*Apus apus*), which become so fat that they frequently weigh more than their parents, can survive at 24°C without food or water for more than 10 days (and this despite frequent disturbances). For the first few days of this starvation they maintain a warm body temperature and lose weight rapidly, but thereafter they drop each night into torpor, then warm themselves up again each morning. During the nightly torpidity their body temperature drops to within a few degrees of the surroundings (ambient temperatures were 19° and 24°C), and oxygen consumption and carbon dioxide production are reduced accordingly. The birds can be handled and probed without arousing them. The adults also become torpid, but not as readily.

Bartholomew, Howell, and Cade (1957) investigated white-throated swifts (*Aëronautes saxatalis*) and found that adults of this species also become torpid after food deprivation. When the birds were kept at 5°C there was some evidence that they would not let their body temperature fall below about 18°C. Furthermore, under the experimental conditions, birds whose body temperature dropped below 18°C died. This suggests that these swifts have only a limited capacity for hypothermia. It is

interesting that their point-of-no-return is close to the temperature from which laboratory rats are unable to warm themselves by their own efforts (Andjus and Smith, 1955).

It is clear that both species of swifts that have been tested are able, under laboratory conditions, to enter torpor and to emerge from it spontaneously. It also appears that they must be "pushed" by food shortage before they will become torpid. This places them in the second category of animals outlined above (stubborn homeotherms). Enough reliable reports have been published about swifts found torpid in the wild so that it seems fairly safe to assume that torpidity does play a role in the energy economy and survival of swifts in nature.

Poor-wills and other caprimulgids. Poor-wills and nighthawks are nocturnal, insect-catching birds with some of the same metabolic problems as swifts: what to do when bad weather cuts off the supply of flying insects? Poor-wills (*Phalaenoptilus nuttali*), the species about which most is known, respond under some circumstances by becoming torpid. In fact, they hibernate. This habit seems to have been known to the American Indians of the Southwest, but not until the reports of Culbertson (1946) and Jaeger (1948, 1949) was the hibernation of poor-wills acknowledged to be firmly established. Jaeger discovered a torpid poor-will in a crypt in a granite cliff, banded it, and found that the same bird returned to the same crypt for three or more winters. Week after week the bird was found torpid at the same spot with a body temperature of 18° to 20°C. Air temperatures during the mornings when the bird's temperature was taken varied from 17° to 24°C. Maximum-minimum temperatures during the same interval at a weather station nearby fluctuated between −6° and 25°C. It is not known how frequently the bird roused itself, if at all, but in view of the fact that it was found to be torpid whenever visited, no matter whether it was day or night, there seems to be little doubt that the torpor lasted for many days consecutively and may have lasted for weeks. Since poor-wills are known to accumulate enormous amounts of fat in the autumn (Marshall, 1955), we must consider the bird to have fulfilled all the requirements of true hibernation.

The poor-will's potential for prolonged hibernation can be estimated. Their metabolic rate in deep torpor at air temperatures lower than 10°C is one-tenth to one-twentieth that of resting poor-wills, and cloacal temperatures are frequently within 0.1°C of the surroundings (Bartholomew, Howell, and Cade,

1957; Howell and Bartholomew, 1959). These authors estimate that ten grams of fat could sustain a torpid poor-will for more than three months if the bird's temperature remained below 10°C.

Marshall (1955) kept poor-wills over the winter in an outdoor cage at Tucson, Arizona, and found that they frequently became torpid. They never remained torpid for more than four days, but this may have been because of frequent disturbances and because of the sunny exposure of the shed in which the birds were kept. There was no series of more than three cloudy days in a row. He found that cold weather alone would not make the birds torpid. It was necessary to withhold food for one or more days before any of the birds would drop into torpor. There is some evidence that poor-wills cannot arouse themselves while the surrounding temperature remains below 15°C (Howell and Bartholomew, 1959).

Brauner (1952) raised a young poor-will and kept it in captivity for many months. Below-freezing cold, starvation, and reduced day-length all failed to push it into hibernation. Its body temperature remained above 36°C during these manipulations. Presumably its hibernation machinery was not yet in readiness.

During an average day, poor-wills have brief periods of activity at dusk and before dawn. Their total period of activity lasts scarcely more than one hour out of 24 (Brauner, 1952). Wild poor-wills collected in the evening during one of their active periods (ambient temperatures between 8° and 30°C) have body temperatures between 40.5° and 43.1°C (Miller, 1950; Brauner, 1952; Marshall, 1955). In between their two periods of activity, however, they sit quietly with body temperature lowered 1° to 3°C. Their temperature is high during exercise, no matter what the time of day or night. This spread between the temperatures of active and inactive or sleeping poor-wills is similar to the spread found in non-hibernating small birds. On some occasions, however, the temperature of resting poor-wills drops considerably lower. Miller (1950) measured the temperature of a wild poor-will flushed from its daytime roost in August at 10:30 a.m. (air temperature 20°C, light rain) and found it to be only 34°C. Although able to fly, this bird obviously was indulging in a bit of the poikilothermia available to this species and no doubt was benefiting from the resultant saving of energy.

In summary, poor-wills are capable of reversible deep torpor and actually engage in true hibernation in the wild. The evidence

accumulated so far suggests that they will not become torpid while food is abundant, and accordingly they are classified as stubborn homeotherms. While they are at rest in the daytime, their body temperature sometimes drops to a level lower than that usually found in other birds.

Marshall (1955) has reported on two trilling nighthawks (*Chordeiles acutipennis*) kept in captivity in an unheated room in Tucson, Arizona, during one summer and autumn. They became quite fat and at the end of November both fell into torpor (body temperatures 18.6° and 19.2°, room 18.7°C) and both emerged from their torpor when warmed. Since nighthawks face the same danger of weather-induced shortage of flying insects as poor-wills, it is not unlikely that nighthawks make use in the wild of the capacity for torpor shown by these captives, but evidence is still lacking.

Hummingbirds. Most birds have no sense of smell and poor night vision — deficiencies that force them to be diurnal. When these deficiencies are combined in the same animal with an extremely high rate of metabolism, as in hummingbirds, the night becomes a dangerously long period of fasting. Presumably in response to the threat of overnight starvation hummingbirds have developed an ability to become torpid. In addition, it is possible that poor weather reduces the supply of nectar and insects on which hummingbirds feed, and that this weather-induced food shortage also exerted a strong selective pressure for torpor on hummingbirds, as it no doubt did on swifts and poor-wills.

Ruschi (1949) noted that many of the hummingbirds in his aviary in Brazil became torpid overnight, some for as long as 14 hours. For nine genera of tropical hummingbirds, average body temperatures at 3 p.m. varied between 39.5° and 44.6° (air temperature approximately 26°C). Body temperatures of sleeping birds at 10 p.m. varied between 36.6° and 40.5° (air approximately 23°C), and the body temperature at which individuals of these species entered torpor varied between 32.0° and 36.3°C. He states that the body temperature must drop about 7°C below the normal daily temperature before the bird becomes torpid.

Anna and Allen hummingbirds (*Calypte anna* and *Selasphorus sasin*) have been studied more than other species. Figure 1 shows the rate of oxygen consumption during a 24-hour period of a captive bird kept at 24°C. The rate of oxygen consumption of the bird when awake but resting was about 14 cc/g/hr (Pearson, 1950). At dusk, however, the rate dropped briefly to a

"sleeping" level and then plunged to a torpid level at about 0.8 cc/g hr. Before daybreak the bird spontaneously roused itself. During the nightly torpor of hummingbirds their body temperature drops to within a few degrees of the surroundings (Pearson, 1950; Bartholomew, Howell, and Cade, 1957). Pearson (1950, 1953) found torpid hummingbirds at night in the wild, so it is clear that this is not just a laboratory-induced torpor.

Since hummingbirds are able to maintain a high body temperature for many hours without food, and yet drop quickly into torpor after dark, even at mild temperatures, they belong in the third category, indifferent homeotherms, members of which can enter torpidity without thermal or hunger stress. In many respects such as this one, the metabolic personality of hummingbirds is like that of the small insectivorous bats.

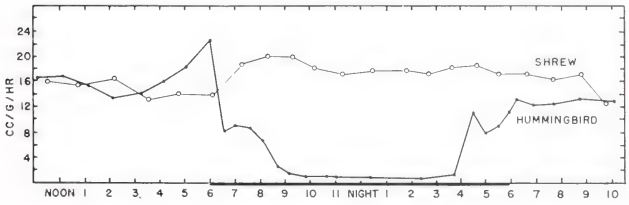

Fig. 1. Rate of oxygen consumption of an Anna hummingbird for 24 hours at air temperature of 24°C compared with the rate for a shrew of comparable size (*Sorex cinereus*). From Pearson (1950).

Some idea of the amount of energy saved by overnight torpidity may be estimated by comparing (Fig. 1) the rate of oxygen consumption of the hummingbird with that of a shrew of about the same size. Both species have about the same rate of metabolism when resting, but the shrew keeps its body warm at all times, an expense that it can afford because its senses enable it to forage for food at all hours. The energy that the hummingbird saves per day by becoming torpid is indicated by the area between the two curves. At cold temperatures the saving would be even greater.

High in the Andes, where a few species of hummingbirds are able to survive, the air temperature at night frequently drops far below freezing, even in the summer. This would endanger torpid birds, but the hummingbirds avoid this danger by going into relatively thermostatic caves at night, and by building their nests in caves (Pearson, 1953).

It is difficult to imagine how nightly torpor could be combined successfully with incubation and brooding of young. Howell and Dawson (1954), by inserting a thermocouple into an Anna hummingbird nest, have shown that the incubating female maintains a high body temperature overnight and that the advanced nestlings also do not become torpid. Obviously, hummingbirds are able under certain favorable circumstances to survive overnight without torpor. The thick insulation of the nest undoubtedly aids them in maintaining a warm temperature. It will be interesting to learn more about the physiological control of temperature in these nesting hummingbirds. Among mammals, body temperatures are elevated during the luteal phase of the estrous cycle and after injection of the luteal hormone progesterone (Landau, Bergenstal, Lugibihl, and Kascht, 1955), and pregnant sloths maintain a tighter control of their body temperature during exposure to cold than do control animals (Morrison, 1945). Perhaps an analagous or even homologous endocrine control operates in birds.

Size × torpidity. A hummingbird, living in the wild, uses about 10.3 Calories per 24 hours if it sleeps at night without becoming torpid, but only 7.6 Calories if it becomes torpid during the night (Pearson, 1954). Why don't more species take advantage of similar metabolic savings? An animal the size of a small bat or a hummingbird can warm itself up from torpidity at a rate of about 1°C per minute (Bartholomew, Howell, and Cade, 1957) so that the entire process takes less than half an hour, sometimes as little as ten minutes. Swifts, which are larger, warm at a rate of about ½°C per minute, and poor-wills, which are larger still, take more than one hour to emerge from torpor. Entrance into torpor takes even longer. Consequently, large birds or large mammals could not afford the time necessary to enter and emerge from torpidity each day in the manner of hummingbirds and bats.

Furthermore, the energy expense of warming up a large mass of tissue is too great. Assuming a specific heat for flesh of 0.95 Cal/kg/°C (calculated from White, 1892), it costs a 4-gram hummingbird 0.114 Calories to warm its body from 10° to 40°C. This is a mere $\frac{1}{85}$th of the total 24-hour energy expenditure of an active hummingbird in the wild. In contrast, a 200-kg bear would need 5100 Calories to warm up from 10° to 37°C — and this is a full 24-hour energy budget for a bear. So even if there were time enough in 24 hours for a large animal to enter and emerge from torpidity, it would be metabolically unprofitable.

Granted that *short* periods of torpor would not be profitable for large birds and mammals, why do they not take advantage of the metabolic savings inherent in *prolonged* periods of hibernation or deep torpor? One reason seems to be that because of their low rate of metabolism they can survive without food for long periods without resorting to torpor. By being relatively inactive, a bear without food can draw for many months on its supply of fat, and a bull fur seal is able to make his supply of fat last during months of vigorous activity while he is ashore during the breeding season. Such large animals clearly do not need to resort to hibernation to survive long periods of starvation.

In conclusion, the occurrence of torpidity among birds is best documented for certain caprimulgids (poor-wills and nighthawks), micropodids (swifts), and trochilids (hummingbirds) – groups usually considered to be fairly closely related taxonomically and evolutionally. The species exhibiting hypothermia are all relatively small birds, and all feed, at least in part, on insects in a manner that exposes the birds to starvation during poor weather. A fourth unrelated group, the swallows, also feeds on flying insects, and numerous field reports make it seem likely that torpidity plays a role in their biology, but physiological measurements are still lacking for them as well as for a few other kinds of birds suspected of torpidity.

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DISCUSSION FOLLOWING PEARSON'S PAPER

STEEN mentioned several other species of birds as possessing the faculty for going into torpor at night. These were the titmouse, the red poll, and the tree sparrow. He also pointed out that these animals arouse (warm up) from this torpor within five minutes after being disturbed, and arouse also when left alone, undisturbed, and in the dark.

PEARSON replied that there are field reports on other birds, but he had mentioned only those on which body temperature changes had been measured. He also indicated that reports on more species surely will appear in the future. There have been many reports of torpid swallows in the wild.

WIMSATT asked if torpidity was correlated with the insectivorous or with the seed-eating habit. PEARSON noted that the seed-eater's food goes through its body in 1½ hours. Thus, dietary factors may be of importance in distinguishing a tendency to hibernate.

STEEN noted the similarity in behavior in aboriginal human beings, for they also are found to be torpid in the morning with body temperatures of 34.5°C. They also cool during the night although they appear to eat well.

DAWE asked if nocturnal torpidity in birds was correlated with season. PEARSON indicated that not enough examples from the wild have been observed. The poor-will seems to be seasonal, the hummingbird shows torpidity throughout the year.

FOLK asked the speaker to comment on the correlation between the cyclic torpidity behavior and oxygen consumption. PEARSON indicated that cyclic behavior in birds was tied in with feeding, and that a burst of activity appeared before the animal went into the torpid state for the night.

VI

ENDOCRINES IN HIBERNATION[1]

By Vojin Popovic
Division of Applied Biology
National Research Council
Ottawa, Canada

In the last few years a number of data have shown hibernation in a new light. Hibernation is an active process which consists essentially of 4 phases: 1) preparation for hibernation; 2) changing the "life speed" from the high to the low one; 3) regulating at the new low level and 4) returning to euthermic life.

If the endocrines have a role in hibernation, and a number of data have suggested it, then the effect of the endocrines is probably to be observed in all stages. However, up to now the most striking manifestation, and therefore the easiest to detect, was the change in the endocrine glands during the preparation period.

Gemelli (1906) was the first to describe the winter involution of the anterior hypophysis in hibernators. Since then, many investigators found involution and hypofunction of the anterior hypophysis, as well as of other endocrine glands, in hibernators. These observations were made during the fall and winter months. Today, it is generally agreed that most endocrines involute before, not after, the hibernator enters wintersleep. The anterior hypophysis (Kayser and Aron, 1938), thyroid (Adler, 1920; Coninx-Girardet, 1927), adrenal cortex (Kayser and Aron, 1950) and gonads (Kayser and Aron, 1950; Kayser, 1955a) change considerably before winter lethargy appears.

Involution of the endocrines before entering hibernation is accompanied by a decrease in the basal metabolic rate (Gelineo, 1938; Popovic, 1951), a decrease in resistance to low oxygen tension of the inspired air (Popovic, 1952a), decreased mobility, and diminished food intake. It is probable that a number of physiological processes are changing during this period. At the end of that period the animal enters hibernation. The environmental temperature is not a decisive factor in this process. Ground squirrels, for instance, will begin to hibernate in autumn

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and will come out of hibernation in spring even if kept in a room at a constant high thermoneutral temperature of 30°C (Popovic and Popovic, 1956).

The appearance of regressive changes of the endocrines before entering hibernation is highly regular. This is why Kayser (1955b) states that there is no hibernation without involution of the endocrine glands.

Kayser (1957a) as well as Eisentraut (1956) consider hibernation essentially as a problem of thermoregulation. However, even if hibernation is considered as a complex problem, a similar approach could be methodologically useful — especially today when data on that problem are not too numerous and often contradictory. This is the reason why attention should be paid also to the possible role of the endocrines in the thermoregulatory mechanism of hibernators as well as non-hibernators. Hibernation is also a kind of adaptation to the cold and other trying conditions associated with cold weather. Therefore, hormonal control of adaptation to cold in hibernators and non-hibernators should also be discussed.

Anterior Hypophysis

A number of experiments, performed on different homeotherms, show that the anterior hypophysis has a considerable role in temperature regulation. It was found that the homeotherm's resistance to cold was greatly reduced after hypophysectomy (Tyslowitz and Astwood, 1942). The inability of hypophysectomized mammals to keep their body temperature at the normal level when exposed to moderate cold is so marked that some investigators consider that such animals are no longer homeotherms (Schaeffer and Thibault, 1946). These findings fitted quite nicely with the picture of hibernators as "lower homeotherms," i.e., hibernators overcome by external cold and passively entering hibernation when the temperature of the environment decreases. Such opinion was widespread even 10-15 years ago and was also one of the reasons why the anterior hypophysis was studied in hibernators. During the summer months, hibernators do not enter hibernation even if the environmental temperature suddenly decreases, because their anterior hypophysis is active and their heat production (measured chiefly by the basal metabolic rate) is as high as in non-hibernators. But when the fall approaches the hypophysis involutes and heat production (BMR) decreases. Soon afterwards, when heat

loss exceeds the decreased heat production, the animal becomes more and more hypothermic and hibernation begins. However, some of the postulates of that theory are not acceptable today and therefore the theory loses some of its validity. Thus, for example, in rats and other non-hibernators both BMR and cold thermogenesis (chemical thermoregulation) are decreased after hypophysectomy (Sahovic, Popovic and Anaf, 1953). On the other hand, in ground squirrels and hamsters, before entering hibernation, the BMR is decreased but cold response is increased when compared with summer animals (Popovic, 1951, 1953; Fig. 1). This fact is quite unexpected: in spite of the involution of the anterior hypophysis and hypofunction of other endocrine glands prior to hibernation, the biggest part of the total heat production (cold thermogenesis) is increased during the cold seasons.

The anterior hypophysis of a hibernator, as well as some other endocrine glands, show a pronounced biological rhythm in the course of a year. The anterior hypophysis involutes in the beginning of the fall (Kayser and Aron, 1950) before any substantial decrease in environmental temperature, and returns to the high spring and summer activity in the middle of the winter, considerably before hibernation ceases (Petrovic and Kayser, 1957). After extirpation and transplantation of the hypophysis, the graft does not show the same rhythm, as judged by its effect on gonads (Petrovic and Kayser, 1957).

The effect of extirpation of the hypophysis was studied in ground squirrels (Foster *et al.*, 1939), and hamsters (Kayser, 1950). Foster *et al.* found that hypophysectomized ground squirrels can hibernate only for 3 days and, unless they were removed from the cold, death occurred.

In our opinion, the hibernation of ground squirrels in the aforementioned experiments is only hypothermia which occurs after hypophysectomy in hibernators as well as in non-hibernators, and which differs in every respect from hibernation (Popovic, 1952b, 1960a) except that the body temperature is low in both cases. Thus, for instance, a non-hibernator, the rat, can stand lethargic hypothermia at 15°C body temperature for 9 hours only, while an artificially cooled ground squirrel can live 119 hours at 10°C body temperature. This long survival of artificially cooled hibernators could be easily misinterpreted as hibernation, as Foster *et al.* (1939) and some other investigators studying endocrines or different physiological processes

did. It is difficult to distinguish hibernation from the hypothermic state in a hibernator. But if, after a few days, hibernation "ends in death" it suggests strongly that only a hypothermic state was involved. The most convincing proof would be obtained by measuring the oxygen consumption which is several times lower in hibernation at the same low body temperature than in hypothermia (Popovic, 1952c, 1959). Even a few hours before death, the oxygen consumption is considerably higher in hypothermia than in hibernation.

Hypophysectomized hibernators rarely survive long enough (Kayser, 1950) to determine whether or not they could hibernate. When exposed to low external temperature, hypophysectomized hibernators soon die, as do the non-hibernators, before it is possible to see if hibernation will occur or not. Our opinion is that similar experiments could be performed now under better conditions. It has been shown recently (Popovic and Popovic, 1956) that normal ground squirrels will hibernate during the winter months even if kept at 30°C room temperature. Similarly, Bartholomew and Hudson (1959) observed torpor in desert ground squirrels during the summer. Thus, it seems that the temperature of thermal neutrality, or slightly lower, is suitable for keeping hypophysectomized hibernators alive for long periods of time and allows the investigator to observe hibernation if it exists.

We conclude that the anterior hypophysis has a temperature regulation role in homeotherms. In hibernators this endocrine gland has a seasonal rhythm but the data purporting to show its possible role in hibernation should not be accepted until performed under better conditions.

Parathyroid

This gland does not seem to have any effect on thermoregulation. At least the parathyroidectomized rats stand the cold as well as the controls (Leblond and Gross, 1943).

There are only a few observations upon the role of the parathyroid gland in hibernation. Adler (1926) thought the parathyroid of hibernating animals was hypoactive, contrary to Skowron and Zajaczek (1947) who saw a hyperactive parathyroid during hibernation, and to Kayser (1957a) who considered it as normally active.

In conclusion, the very few collected data about the parathyroid gland in hibernators do not indicate any decisive role of this endocrine gland in hibernation.

Thyroid

A number of data show that the thyroid has a definite role in the thermoregulation of non-hibernators.

In anesthetized rats (Gergely, 1943) as well as in unanesthetized rats (Schaeffer, 1946), the heat production is decreased after thyroidectomy. The decreased heat production is probably the cause of somewhat diminished cold resistance found

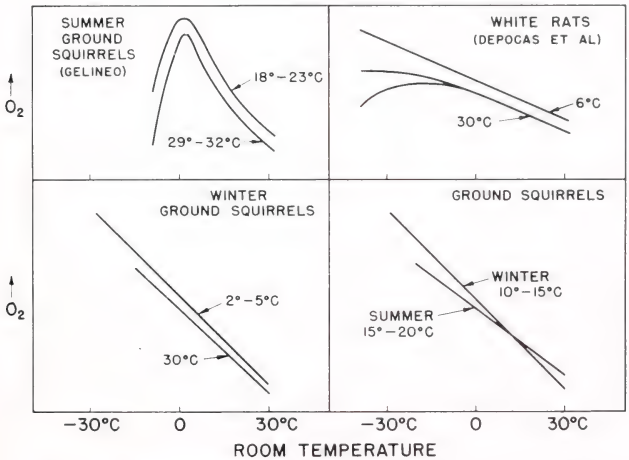

Fig. 1. Oxygen consumption of active ground squirrels. *Upper left,* active summer ground squirrels adapted to 18-23° C and 29-32° C. *Upper right,* white rats adapted to 6° and 30° C. *Lower left,* active winter ground squirrels adapted to external temperature of 2-5° C or 30° C. *Lower right,* active summer and winter ground squirrels adapted to similar external temperatures.

after thyroidectomy (Leblond and Gross, 1943) or after administration of thiouracil (Ershoff, 1948). After thyroidectomy the basal metabolic rate is diminished but the cold response *per se* (cold thermogenesis, i.e., maximal metabolic rate of a homeotherm exposed to cold minus basal metabolic rate) is not altered (Sahovic and Popovic, 1953). The drop of BMR in thyroidectomized animals is considerable, up to 30-40 per cent (Popovic

and Popovic, 1951). A similar difference in BMR is found between cold- and warm-adapted rats (Depocas *et al.*, 1957) (Fig. 1).

Boatman (1959) concluded that the thyroid has a role in maintaining body fluids in an efficient equilibrium for rapid adjustments in a cold environment.

Thyroid hormones increase the 0_2 consumption of tissue slices too (Barker, 1951, 1955); after thyroidectomy the O_2 consumption of tissue slices is 30 per cent smaller (Spach *et al.*, 1955). However, it is not certain how thyroid hormones affect metabolism, whether centrally or not. The vegetative nervous system as well as adrenaline could play a role.

Thyroxin or thyroidectomy affect the level and activity of some enzymes (Tipton *et al.*, 1946; Tipton and Nixon, 1946; Maley, 1957).

The thyroid activity of homeotherms is increased during cold exposure as shown by histological investigations (Uotila, 1939) or by incorporation of iodide into the thyroid gland (Leblond *et al.*, 1944; Cottle and Carlson, 1956). In cold adapted non-hibernators the increased activity of the thyroid (over twice that of warm adapted as calculated by Hart, 1958) is sustained for long periods of time (Cottle and Carlson, 1956; Woods and Carlson, 1956). The thyroid is also the seat of seasonal changes independent of environmental temperature (Bernstein, 1941).

The activity of the thyroid gland is greatly reduced when deep hypothermia is induced in non-hibernators, as measured by the histological picture (Ariel and Warren, 1943) or by incorporation of I^{131} (Verzar and Vidovic, 1951; Andjus *et al.*, 1954).

In hibernators the thyroid gland changes considerably from season to season. The changes are even greater than in non-hibernators and in contrast with non-hibernators the cold seasons induce regressive changes, in hibernators, as illustrated by the histological picture (Adler, 1920; Coninx-Girardet, 1927; Kayser and Aron, 1938; Zalesky and Wells, 1940), blood studies (Kratinov and Skirina, 1947), or by the incorporation of I^{131} (Lachiver, 1952a,b; Vidovic and Popovic, 1954). The incorporation of I^{131} into the thyroid of active ground squirrels is very low in autumn, hardly higher than in hibernating animals at a body temperature of 5-10°C (Vidovic and Popovic, 1954). In active hibernators prolonged cold exposure does not change the histological picture of the hypoactive thyroid gland (Kayser, 1939; Deane and Lyman, 1954) except in the midwinter cold

(Suomalainen, 1956) but increases the basal metabolic rate (Popovic, 1955a; Adolph and Richmond, 1956). Since the increase or decrease of BMR, a consequence of acclimatization to cold or warm environments, is present in thyroidectomized guinea pigs (Popovic and Popovic, 1951), it seems that the adaptive changes of BMR are not to be associated with thyroid gland. In the middle of the winter the incorporation of I^{131} returns to the higher level (Vidovic and Popovic, 1954). Thus in active ground squirrels the thyroid becomes active again, considerably before the period of hibernation ceases. These findings were confirmed on other hibernators (Lachiver *et al.*, 1957). However the reactivation of thyroid is accompanied by more frequent awakenings and shorter hibernating periods.

Total extirpation of the thyroid does not prevent ground squirrels from hibernating (Foster *et al.*, 1939). The frequency and the length of periods spent in hibernation are similar to that of the controls (Popovic, 1955a). The same results were observed after prolonged administration of thiouracil (Popovic, 1955a). It was mentioned that the basal metabolic level of active hibernators is low during autumn and winter. A further lowering of BMR was observed when hibernators (ground squirrels) were exposed during the cold seasons to the temperatures of their thermal neutrality (30-32°C) or treated with thiouracil (Popovic, 1952b). These tests show that although the involuted thyroid is not essential for entering hibernation it has a certain role in keeping the BMR at the winter level.

Injected thyroid extracts wake up the hibernating animal (Adler, 1926). Hibernation of hedgehogs stops when thyroid extracts of either winter or summer hibernators are used (Uiberall, 1934). Injected thyrotropic hormones decrease the number of days spent in hibernation (Foster *et al.*, 1939) and injected homogenates of the anterior hypophysis stimulate the thyroid during the cold seasons but not in summer (Petrovic and Kayser, 1958).

Seasonal changes of many endocrine glands, their involution during hibernation, and particularly his own results (which indicated that injected extracts of a number of endocrine glands wake up hibernators) gave Adler one of the basic postulates for developing his endocrine theory of hibernation. However, the experimental basis of Adler's work often has been submitted to strong criticism. It has been said that Adler was not careful about the right temperature of injected solutions, that thyroid and other endocrine gland extracts were not properly prepared,

that they had very small or no hormonal content, etc. The most important objection is that the glandular extracts had to be injected by needle, and this technique evidently is not appropriate. It is known that the nerves of hibernators conduct impulses even when the body temperature is very low (Chatfield *et al.*, 1948). The injection by needle will wake most hibernating animals even if no substance or only a physiological solution is injected. Even smaller stimuli interrupt hibernation, such as measurements of colonic temperature by a small thermometer or handling of the animal, etc. For this reason Lyman and Chatfield (1956) stated that "more delicate and better controlled methods must be developed before the cause of spontaneous arousal is clarified."

However, the new technique of permanent cannulation of the aorta and vena cava in small laboratory animals (Popovic and Popovic, 1960) now permits clarification of this question. The hormones of any gland can be injected into the blood stream of the hibernating animal without disturbing normal winter-sleep; thus all these questions about hormones and their effect on hibernation could be investigated now under better experimental conditions.

Feeding the hibernators with dry thyroid gland results in cessation of hibernation (Adler, 1926). Moreover, a very small amount of that gland given with the food every third day gives the same effect. Elevation of the BMR is very small in these experiments. Values of the BMR do not exceed those in summer in the same animals (Popovic, 1955a) so that the toxic effect of thyroxin probably should not be considered at all as the cause of cessation of hibernation.

In summary, the thyroid gland in hibernators shows a strongly accentuated biological rhythm. In contrast with non-hibernators, cold seasons induce hypofunction of thyroid in hibernators. Without the thyroid, hibernators can continue to hibernate the same as with a slightly activated gland. But hibernation will stop if the thyroid is very active — for instance, when the BMR is at high spring-summer level.

Pancreas

It is not known if insulin has any appreciable role in the temperature regulation of non-hibernators. However, when injected in massive doses, insulin induces a marked lowering of the level of blood sugar and of the body temperature. The drop in body

temperature, at least in man, is a consequence of increased heat loss while heat production remains the same (Lundback and Magnussen, 1941).

Heat production in alloxan diabetic rats was found to be normal (Sahovic *et al.*, 1952). Injected insulin increases the amount of glycogen in intrascapular fat tissue of hypophysectomized rats (Engel and Scott, 1950) and it could be that it has a similar effect in winter hibernators, with an involuted anterior hypophysis.

In artificially cooled rats, non-hibernators, the level of blood sugar was very low when lethargic hypothermia was sustained for several hours (Popovic, 1955b). However, the hypothermic rat cannot be revived by rewarming. In artificially cooled ground squirrels with stabilized body temperatures at 10°C the level of the blood sugar tended to decrease all the time. After 100 hours, blood sugar values were 40 mg %, similar to that in hibernation where hypoglycemia is usually observed (Bierry and Kollmann, 1928; Ferdmann and Feinschmidt, 1932; Agid and Popovic, 1957), but in hibernators as well as non-hibernators death ensued when the animals were rewarmed.

Because insulin injected in large doses decreases the body temperature of a homeotherm, non-hibernator or hibernator, investigators used this hormone in trying to produce artificial hibernation. Dworkin and Finney (1927) reported that insulin and moderate cold induce artificial hibernation in woodchucks. Suomalainen (1939b) found the same to be true in hedgehogs using Mg and insulin, and Suomalainen and Petri (1952) produced the same results in hamsters using insulin only. A similar technique was used to induce deep hypothermia in non-hibernators (Giaja *et al.*, 1955; Popovic, 1960b) and in hibernators (Kayser, 1955b).

As for most endocrines, cyclic changes have been observed in the pancreas of hibernators (Skowron and Zajaczek, 1947). It was also shown that the relationship between B and A cells differs from season to season (Skowron and Zajaczek, 1947; Aron and Kayser, 1956).

We conclude that there is not enough evidence to assume that the pancreas has a role in hibernation. What was called artificial hibernation in hibernators is, in all likelihood, only induced hypothermia. Since hibernators withstand induced hypothermia longer than non-hibernators, this state was misinterpreted as hibernation.

Gonads

Some investigators (Hemingway, 1945) consider the gonads as one of the endocrine glands which controls body temperature. But even if gonads have a part in the temperature regulation of homeotherms, it is probably small. Thus, for instance, the body temperature of homeotherms is not changed after extirpation of the gonads.

Involution of the gonads is very pronounced before and during hibernation (Kayser and Aron, 1950; Kayser, 1955a; Lyman and Chatfield, 1956). Foster *et al.* (1939) were convinced that, among other endocrine glands which have a definite role in hibernation, gonads play a part too. These investigators found an inverse correlation between hibernation and activity of gonads, as well as a "resistant state" to hibernation when gonads are active. In the second part of the winter, castrated animals will continue to hibernate more often than controls. At that time of the year a small activation of the gonads of hibernators may be seen. However, hibernation is observed after administration of anterior hypophysis hormones and a consequent activation of the gonads. Similarly, Lyman and Dempsey (1951) showed that after testosterone was injected golden hamsters could continue to hibernate.

Some hibernators, for instance ground squirrels, breed only once a year. The breeding period is usually immediately after arousal from hibernation. Bats are in a state of prolonged oestrus during the winter (Guthrie and Jeffers, 1938). Ovulation of follicle and implantation of the egg occur in April (Wimsatt, 1944). The response of reproductive organs of winter bats to injected gonadotrophic hormones is similar to reactions found in immature or hypophysectomized non-hibernators (Sluiter *et al.*, 1952).

In conclusion, the gonads of hibernators are sites of profound seasonal changes. However, hibernation occurs not only in autumn and the first part of the winter when the gonads are involuted but also in the last part of the winter when the gonads show some activity. Also, activation of the gonads after administration of anterior hypophysis hormones or injection of testosterone is compatible with hibernation. Therefore, it seems that the gonads do not exert any major effect on the process of hibernation.

Adrenal Medulla

The cold resistance of homeotherms is greatly diminished after extirpation of the adrenals (Schaeffer and Thibault, 1945b). After demedullation, the cold resistance is diminished in white rats (Ring, 1942) but not in dogs (Morin, 1942). Greatly decreased cold thermogenesis after adrenalectomy in white rats is chiefly restored after administration of desoxycorticosterone, DOC (Sahovic *et al.*, 1951b). Cold thermogenesis is not appreciably changed after adrenalectomy if the rats are carriers of cortical grafts (Sahovic *et al.*, 1951a).

A considerable number of investigators failed to find a calorigenic effect of adrenaline (see Griffith's review, 1951), but the recent data on high calorigenic action of adrenaline are becoming more and more convincing (Griffith *et al.*, 1940; Morin, 1943). Adrenaline and noradrenaline are hormones which cause an immediate metabolic reaction. The calorigenic effect of adrenaline is higher in rats with an active thyroid, after long cold exposure (Ring, 1942; Hsieh and Carlson, 1957). Calorigenic action of adrenaline is small in animals which have a low BMR as a consequence of adaptation to the temperature of thermal neutrality (Schaeffer and Thibault, 1945a). After thyroidectomy the adrenaline effect is small; after thyroxin treatments it is high (Issekutz and Harangosó-Groszy, 1942). The calorigenic effect of noradrenaline is also increased in cold-adapted animals but not in warm-adapted ones, either anesthetized (Hsieh and Carlson, 1957) or unanesthetized (Depocas, 1960).

In rats (Fisher *et al.*, 1955) and dogs (Malmejac *et al.*, 1956), when cooled below 20°C body temperature, the adrenal medulla is no longer activated by cold.

During hibernation the adrenal glands of some hibernators seem to be involuted. The involution is very pronounced, up to 50 per cent of the volume of the glands (Valentin, 1857), but in the hibernating hamster the weight of the adrenals is not changed (Holmes, 1955). Some investigators found that the adrenal medulla is involuted (Suomalainen, 1938b, 1940), while others did not find medullar involution (Kayser and Aron, 1950).

The adrenaline content of the adrenals is increased during hibernation, while noradrenaline content is decreased (Suomalainen and Uuspää, 1958). It was suggested (Kayser, 1940) that the highly variable content of adrenaline in the adrenal medulla of winter hibernators could be associated with the frequency of arousals from hibernation.

The medullar hormones probably play an important role in arousal from hibernation. Chatfield and Lyman (1950) found an increased effectiveness of sympathetico-adrenal activity during that process. Even if eviscerated the hibernating animal shows some ability to rewarm.

Adrenalectomized ground squirrels with cortical grafts are able to hibernate and to wake up as do control animals (Popovic, 1952b). This suggests that if calorigenic hormones, adrenaline and noradrenaline, have a role in hibernation, especially during arousal, then the medulla is not their only source.

In conclusion, it seems that the adrenal medulla in hibernators shows certain biological rhythms. It seems also that without this gland, hibernators may enter hibernation and wake up normally.

Adrenal Cortex

Like some other endocrines the adrenal cortex has a role in temperature regulation. Cold resistance is highly reduced in cortico-deficient rats. Treatment with cortin or with DOC partially restores cold resistance (Tyslowitz and Astwood, 1942; Sahovic *et al.*, 1951b). The cold resistance of a normal man is increased after DOC treatment (Zarrow, 1942).

After exposure to a cold environment the cortical function seemed to be increased only during the first days of exposure (Levin, 1945). Adrenal cortical functions return to the previous level of activity after a few days as judged by incorporation of P^{32} (Nicholls and Rossiter, 1955, 1956). Similarly, it was found that adaptation to cold does not increase the need for cortical hormones (Sellers *et al.*, 1951; Heroux and Hart, 1954). The homeotherms are able to adapt to cold environment without the adrenals (Sellers *et al.*, 1951; Heroux, 1955) as well as without thyroid (Popovic and Popovic, 1951). However, in hibernators, some investigators could not find the manifestation of adaptation to cold even after partial extirpation of these glands (Kayser, 1939).

In induced hypothermia of non-hibernators the output of ketosteroids from the adrenals is decreased (Ganong *et al.*, 1955). After rewarming from hypothermia, dogs showed stress manifestations (Sarajas *et al.*, 1958).

In hibernators, the cortex shows similar biological cycles to the thyroid, gonads or the anterior hypophysis. The adrenal cortex is involuted prior to hibernation (Kayser and Aron, 1950). Similar involution of the adrenal cortex has been ob-

served in non-hibernators after hypophysectomy. Suomalainen (1938b, 1940), too, saw pre-hibernal involution of the cortex in hibernators. Later, however, Suomalainen (1954) concluded on the basis of his new observations that the cortex of hedgehogs is hyperactive during cold seasons. The same author was able to confirm these findings in hamsters (Suomalainen and Granström, 1955). He concluded that the "alarm reaction" of Selye is present in hibernators during cold months. Zalesky and Wells (1940) also observed a well developed cortex in ground squirrels exposed to the low environmental temperature.

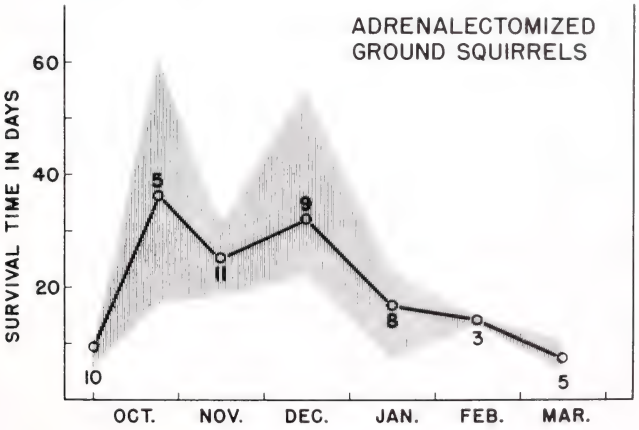

Fig. 2. Survival time of adrenalectomized ground squirrels during autumn-spring period. From Popovic and Vidovic (1951).

Survival of summer-active hibernators after adrenalectomy is similar to that of non-hibernators. But during the winter, survival time of adrenalectomized marmots (Britton, 1931; Britton and Silvette, 1937) and of adrenalectomized ground squirrels (Popovic and Vidovic, 1951; Vidovic and Popovic, 1954) was much longer. This contrasts with the non-hibernators in which cold decreases the survival of adrenalectomized animals. Figure 2 shows the survival of adrenalectomized ground squirrels during the cold months. This is similar to Kayser's graph (1952) showing the time spent in hibernation during the same

period of time. The adrenalectomized ground squirrels live longer during the cold seasons but none of them enter hibernation (Popovic and Vidovic, 1951; Vidovic and Popovic, 1954). However, the adrenalectomized ground squirrels hibernate either when a small cortical fragment of one of the adrenals is left *in situ* (Vidovic and Popovic, 1954) or with a cortical graft in the anterior chamber of the eye (Popovic, 1952b). Treatment with cortical hormones was also able to restore the ability to hibernate in adrenalectomized ground squirrels, sometimes after 2-3 days of DOC or cortisone injection (Popovic *et al.*, 1957). Some of these results were confirmed on another hibernator, the hamster. Adrenalectomized hamsters hibernate ten times less than the controls (Kayser, 1957b). In a repetition of the same experiments on the same species, Kayser found that none of the adrenalectomized hamsters would hibernate (Kayser and Petrovic, 1958) unless treated with cortisone or, even more readily, with DOC.

Hibernating Gland

Up to now the hibernating gland did not justify the name given it by Vignes in 1913. At present it is not known if the hibernating gland has any role in hibernation. It is not known if it is an endocrine gland, and all research has failed, up to now, to extract from that tissue any active principle. However, it is known that hibernating gland tissue is also found in non-hibernators (Wertheimer and Shapiro, 1948).

The hibernating gland is a fatty, reserve tissue, brown in color and situated between the scapulae. The respiration of this tissue is much higher than that of other adipose tissues, and the decrease of respiration is smaller during cooling (Hook and Barron, 1941). Some investigators found that injected extracts of this gland into active hibernators decrease the metabolic rate and the body temperature (Wendt, 1943); but the same effect is found for the extracts of other tissues even in non-hibernators. Moreover, Klar (1941) was not able to confirm the findings of Wendt.

The hibernating gland shows a rhythm similar to certain endocrine glands. During the winter it is involuted (Valentin, 1857), but Suomalainen and Herlevi (1951) concluded on the basis of their observations that the hibernating gland has a role in the "alarm reaction." Immediately after arousal the hibernator is in a state of stress.

In conclusion, the hibernating gland is a reserve tissue of high activity showing strong seasonal changes. For the moment we do not have sufficient information to understand its role in hibernation.

Summary

After half a century, the endocrine theory of hibernation offers little more than it did when first postulated. The data are often contradictory and at times obtained by using techniques not particularly appropriate. In spite of this, there are positive indications that some of the endocrines play a role in hibernation.

The data presented here suggest that the endocrine glands have the same function in hibernators and non-hibernators as far as temperature regulation is concerned. But there are exceptions. For example, in active winter hibernators exposed to cold there is no increased activity of the thyroid and other endocrines. Adrenalectomized ground squirrels and marmots survive winter temperatures longer than summer temperatures.

Most of the endocrines involute prior to hibernation and resume nominal functioning in active winter hibernators even before winter sleep finally ends. Hibernators in which the activity of the endocrines, especially the thyroid, has been artificially increased either do not hibernate at all or hibernate less than the controls. However, reports in recent years indicate that involution and hypofunction of some endocrines, particularly the adrenals, do not occur before or during the hibernation period.

Adler's experiments on injections of endocrine extracts and hormones are, for the most part, unacceptable today because of inadequate experimental techniques. A new technique is suggested here.

Using the extirpation and hormone replacement method it was shown that without the adrenal cortex or its hormones, ground squirrels and hamsters do not hibernate. Both desoxycorticosterone and cortisone restore the ability to hibernate in adrenoinsufficient animals, but to different degrees. Adrenalectomized ground squirrels with cortical grafts in the eye hibernate normally.

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DISCUSSION FOLLOWING POPOVIC'S PAPER

ZIMNY opened the discussion by pointing out three endocrinological observations she has made on the tissue of hibernating animals:
(a) Using the Sudan-Black B reaction for lipid and the Schultz reaction for cholesterol, she found that these materials increased in the adrenal cortex during hibernation. In this connection she believes the material is stored there to be mobilized for use in arousal, and not for use as a slowly releasing material during hibernation.
(b) Using the chromaffin technique she found an increase in adrenalin in the adrenal gland during hibernation.
(c) Upon staining the pancreas, she found an increase in granulation of the beta cells during hibernation.

SUOMALAINEN indicated that he, too, had observed an increase of adrenalin in the adrenals and observed that beta cells of the pancreas were proportionately more numerous in hibernating animals than in non-hibernating animals.

POPOVIC asked SUOMALAINEN if injection of insulin could produce hibernation in the summer animal. SUOMALAINEN replied that injection of insulin without magnesium salts puts the animal into an "artificial hypothermic state."

ZIMNY said she had heard of an interesting case of a diabetic man in the Arctic who found he did not require insulin while doing field work. She speculated as to whether, therefore, cold alone might not produce an increased beta cell population.

WIMSATT asked what results were obtained using radioactive analysis of thyroid function. POPOVIC said his tests by this means show some thyroid activity. KAYSER said that his experiments with hamsters using I^{131} showed him that neither the uptake nor the release of I^{131} measured by counts with the Geiger-Muller apparatus could provide a correct evaluation of thyroid activity. WIMSATT remarked that he also had tried such a study with bats, and could get no sense out of the results. He (WIMSATT) concluded that conditions were not standard enough, and that the bat is more easily prodded to arousal than other hibernating animals, hence acquisition of data on hibernation in the bat is far more difficult than in other species. JOHANSSON stated that the protein-bound iodine in the hedgehog is decreased during hibernation as compared with the situation during the summer. JOHANSSON asked if infection occurred in the preparation in which a cannula was permanently imbedded in a vessel. POPOVIC replied that they had both arterial and venous cannulae in vessels of ground squirrels longer than 40 days without infection. LYMAN volunteered the information that he had used arterial cannulae, and animals had carried these devices as long as four months without infection.

VII

HISTOLOGICAL CHANGES DURING THE HIBERNATING CYCLE IN THE ARCTIC GROUND SQUIRREL[1, 2]

By William V. Mayer
Department of Biology
Wayne State University
Detroit 2, Michigan

On the Arctic slope of Alaska, where the field work was done for this particular research problem, the Arctic ground squirrel is active from May until October and hibernates during the rest of the year. The animal completes its life cycle during these brief months. It awakens from hibernation early in May and mates almost as soon as it emerges from its burrow. The young are born in mid-June after a 25-day gestation period and are self-sufficient by mid-July. They attain approximately adult weight and are ready to enter hibernation by the latter part of September or the early part of October.

On the flat, wet, treeless Arctic slope, one would hardly expect to find a burrowing animal. However, on the few high spots of ground which stand above the normally wet tundra, the permafrost table is low enough to allow these animals to burrow beneath the surface and be protected from the full fury of winter (Plate 1, fig. 1).

Hibernation is not a continuous process involving entering the torpid state in September followed by a single emergence only in May. Periodically, during the winter, the animal awakens at intervals varying from two to three weeks. The typical hibernation pattern consists of a relatively slow drop of temperature as heat radiates from the body, a rather consistently low body temperature during hibernation, and an almost explosive awakening which the animal undergoes during a relatively short period of time. The temperature at which hibernation takes place is correlated with that of the environment, and it is possible to have animals hibernating at body temperatures varying from 0° to 19°C.

[1] This research was supported, in part, by the United States Air Force under Contract Number AF 18(600)-843, monitored by the Arctic Aeromedical Laboratory, Alaskan Air Command, Ladd Air Force Base, Alaska.

[2] Contribution Number 39 from the Biology Department of Wayne State University, Detroit 2, Michigan.

Due to the profound effect of temperature on metabolic processes it is essential to know the body temperature at which an animal hibernates. After taking rectal temperatures on 55 different individuals over a period of a year, the 425 records thus obtained on animals considered to be warm and active showed the average body temperature to be 36.4°C. The environmental temperature for this period averaged about 19°C. These captive animals in the laboratory were used to being handled and, while never becoming completely resigned to the taking of rectal temperatures, did not struggle or fight as much as animals caught in the field. Seventy-five rectal temperatures taken on squirrels captured in the field during July and August showed an average temperature of 39.1°C, the heightened temperature in all probability due to the fear which the newly caught animals evidenced on being trapped and handled.

In addition to animals kept at an average environmental temperature of 19°C, six animals were kept at a more or less constant temperature of 11.4°C. Thirty-nine rectal temperatures taken throughout the year on these six warm and active squirrels averaged 34.8°C or only 1.6°C less than the body temperatures of those kept at 19°C. In five warm and active animals kept at an average temperature of 1.6°C, a total of 43 rectal temperatures taken throughout the year averaged 36.1°C, or only three-tenths of a degree centigrade less than that of the animals kept at 19°C. Thus, the average body temperature of the warm and awake squirrel varies slightly with environmental temperature, and also between captive and wild animals, although the latter difference is probably due to the difference in reaction to being handled. The minor rise in body temperature as the environmental temperature approaches 0°C is indicative of an increase in metabolism necessary to remain warm and active.

Among animals in hibernation, a similar series of measurements was made. Thirty rectal temperatures on 25 different animals, hibernating in an environmental temperature of 19.7°C, averaged 18.5°C. In an environmental temperature of 18.4°C, 28 rectal temperatures on 20 different animals averaged 16.6°C. Thirty-three rectal temperatures on six different animals, hibernating in an environmental temperature of 11.4°C, averaged 13.7°C. Twenty-five rectal temperatures of five different animals, hibernating in an environmental temperature of 1.6°C, averaged 4.2°C. The body temperatures during hibernation below those of the environment occur only at relatively high environmental temperatures and probably are due to heat loss

through respiration and evaporative cooling from the surface of the squirrel.

The typical picture of an animal in hibernation at temperatures above 0°C was determined by the use of a rectal thermistor inserted to a depth of 12 cm. The thermistor was connected to a continuous recording milliammeter. The pattern thus obtained, as typical, was of an initial rise at the insertion of the thermistor and then a slow drop of the body temperature (Fig. 1). As the decrease begins, it is more rapid than after the body

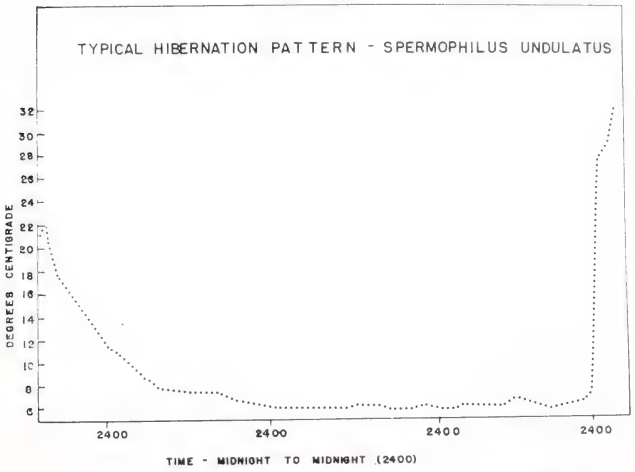

Fig. 1. Arctic ground squirrel hibernation pattern. Slow loss of heat from body, relatively consistent low temperature during hibernation, and explosive awakening from hibernation are typical.

temperature has been lowered to about 11°C. In the typical picture, the time taken for the temperature to drop from 22° to 11°C was 10 hours. The time necessary for the temperature to be lowered from 11° to 6°C was 24 hours. The animal then remained at about this temperature for a period of up to three weeks before awakening. The temperature pattern at the awakening is explosive.

Upon awakening from hibernation, the temperature first rises from 4° to 17.5°C in one and one-half hours, and then takes the

next one and one-half hours to rise from 17.5° to 32°C (Fig. 2). Accompanying this rapid rise in body temperature, there is also a typical awakening pattern of behavior. At the hibernating body temperature, there are about three irregular respirations per minute. The heartbeat is not noticeable at 5°C even with a diaphragm stethoscope. An investigation of the heartbeat pattern reveals that the animal in hibernation has so slow a heart action that the heart operates by slowly wringing; too slow to produce the characteristic sounds heard through the chest wall of the normally awake and active animal.

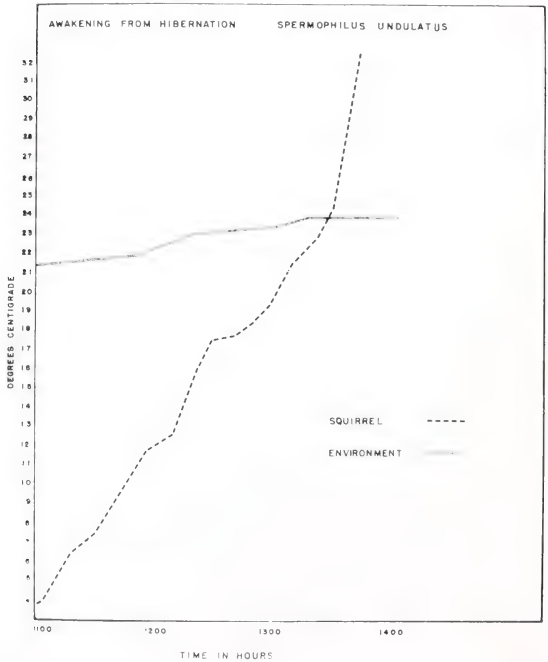

Fig. 2. Temperatures during awakening from hibernation. The steep rise between 12°C and 17.5°C is occasioned by the shivering of the animal. The second steep rise, at 24°C, marks the time the animal opens its eyes and sits up.

The behavior pattern is also characteristic, and certain actions can be correlated with temperature. By the time the temperature has reached 11°C, the increasing respiratory rate as indicated by chest movements is obscured by other movements of the body. At 12°C, the first shivering reaction sets in, with a corresponding rise in body temperature. About this time, the hind limb motions begin. When a temperature of 16°C has been reached, the animal tries to right itself. At a temperature of 17.5°C, the shivering stops and the animal begins to move its tail. At 24°C, the squirrel opens its eyes and suddenly sits up. At this time, a second, more rapid temperature rise is noted. By the time the body temperature has reached 25.4°C, the animal is sitting up, flicking its tail and is considered essentially warm and active.

The problem of the temperature patterns of animals exposed to temperatures of less than 0°C and whose body temperatures drop below 0°C was explored. Twelve animals awakened when the body temperature reached 0°C. Seven animals, exposed to −15°C, either awakened or froze. One survived in this temperature for 50 hours without the protective insulation of a nest. While several animals recorded body temperatures in hibernation below 0°C, only two dropped below −3°C. These two animals had been repeatedly stressed by prolonged exposure to cold and by at least three awakenings from hibernation prior to the below zero body temperature experiments. They attained body temperatures of −5.5°C and −4.6°C, respectively, before the formation of ice in the body was indicated by a rapid rise of the rectal temperature to 0°C (Fig. 3). This work confirms the experiments of Kalabukhov (1935) who supercooled a mouse to −2.2°C and a bat to −7.5°C before ice formation occurred.

In a natural environment, the animal protects itself by the manufacture of a large nest of dried leaves, grass, lichens, moss, and hair in a burrow system constructed in such a fashion as to face away from prevailing winds. The snow cover in winter varies from negligible to a depth of about 92 cm. In order to determine the temperatures to which the animal would be exposed during hibernation, thermistors were introduced into burrows on 25 foot cables fastened to the ground squirrels by a harness made of cloth bandage. The squirrels were chased into the burrows and the cable payed out behind them. The cable would be taken into burrows for a distance of from 10 to 22 feet. Any cable taken in less than 10 feet would be withdrawn and the experiment repeated. The ends of the cable protruding from

the burrow were attached, under inverted cans, to poles mounted in the ground by the burrow entrance. They were tagged to identify them and were read on the average of every two days during the winter so that the weekly averages are those of three temperatures in most cases. This experiment was conducted from September 12, 1954 to May 7, 1955, the period approximating the time from entrance into hibernation in the fall to the time of emergence in the spring.

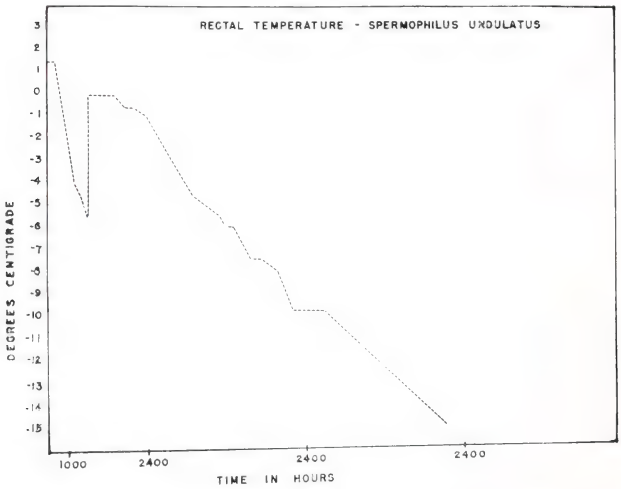

Fig. 3. Temperature of an animal supercooled to −5.5°C. The rapid rise in temperature is occasioned by ice formation within the tissues.

The thermistors were recovered by digging out the burrows the following spring as soon as the ground had thawed enough for this purpose. The deepest penetration beneath the surface was found to be 3.8 feet; the shallowest, 1 foot. For all thermistors, the depth averaged 1.72 feet. During the winter, the variable snow cover averaged 59 cm.

During this same period, the maximum and minimum environmental temperatures were recorded. There was a 100° Fahrenheit range in the temperature during this period from a high of 58°F to a low of −42°F, the average environmental temperature

being 22.8°F. The burrow temperatures varied during this period from 36.5°F to 16°F with a range of 20.5 F degrees to average 25.2°F for the season, which is 2.4° higher than the environmental average. The average temperature for the warmest burrow was 26.1°F and that for the coldest 23.7°F (Fig. 4).

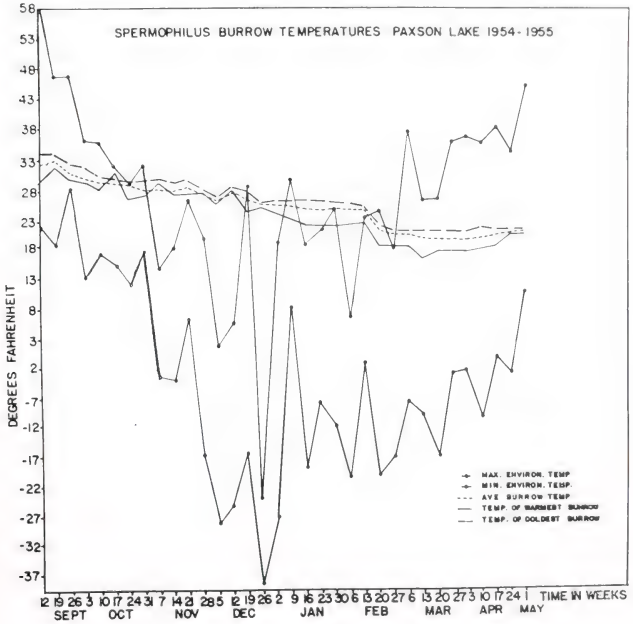

Fig. 4. Comparison of burrow temperatures with those of the environment. Paxson Lake, Alaska.

In correlating these temperatures with snow cover, it was found that the burrow covered with the greatest depth of snow had a temperature range of from 32 to 21°F or 11 F degrees and an average for the winter of 25.8°F. The burrow with least snow covering, in which the bare ground could be noted during the winter, was occupied all winter. The thermistor was 30 cm beneath the surface of the ground, and the temperature here ranged from a high of 31.7°F to a low of 16.5°F or through

15.2 F degrees, for a season average of 23.7°F. In contrast, in another burrow in which the thermistor was likewise 30 cm beneath the surface of the ground the temperature ranged through 18 F degrees from a high of 34° to a low of 16° for an average of 23.7°F, or the same as for the above, even though this burrow was covered by 87 cm of snow. It is significant that the temperature on any one day in all the burrows never varied one from another by more than 5°F. The seasonal average varied only 2.31°F from the highest to the lowest burrow temperatures despite the difference in depth of the thermistors beneath the surface and the difference in snow cover over the burrows. It is thus concluded that the depth of snow cover and the distance of the burrow beneath the surface, in excess of 30 cm, does not give appreciably greater protection to the hibernating squirrel than a shallow, less well covered burrow.

Wherever the environment makes it possible, the burrows tend to be oriented with their openings away from the prevailing wind and in areas of favorable drainage. Burrow temperatures during the winter are well above the environmental minima and show relatively little fluctuation. In the wild, burrow temperatures do not drop below a minimum of 16°F which is considerably higher than the temperatures at which the animals have been kept in the laboratory for a long period of time. From this, it is apparent that a simple hole in the ground would give a great deal of protection from the cold. When this is coupled with the large and well insulated nest, the combination makes it possible for the Arctic ground squirrel to maintain itself through the severe Arctic winter.

Histological Changes

The effect of hibernation upon the various tissues of the body is a subject of considerable interest. In general, it might be said that all of the tissues of the body apparently involute during the period of hibernation. If we begin with the digestive tract, there have been references in the literature to the fact that the digestive tract and the exocrine pancreas are involuted during hibernation. Valentin (1857-58) and, recently, Lyman and Leduc (1953), and Mayer and Bernick (1957) indicated the changes that take place in the digestive tract of various animals during hibernation. The lining of the stomach of the warm and active Arctic ground squirrel, stained by the periodic acid Schiff (PAS) method, shows mucoid material limited to the

surface epithelial cells and a few of the superficial mucous neck cells. However, after the squirrels are in hibernation for a period of three weeks, the stomach lining shows an increase in the amount of mucus stored in the superficial cells. It has been shown by others that the golden hamster, still containing food in the esophageal portion of its tract, likewise will show the presence of neutral or slightly acid mucus. Concurrently, with this increase in mucus in the superficial cells, the chief cells also contain mucoid material. After three months of hibernation, there is an even more marked increase in mucus with the superficial cells, the mucous neck cells, and the chief cells filled with PAS-positive material. However, within twenty-four hours after awakening from hibernation, the stomach lining displays a marked loss of mucoid material and mucus is restricted to the cells of the surface epithelium and the underlying mucous neck cells.

The parietal and chief cells, which can be seen plainly in the stomach wall of normally warm and active squirrels, become less obvious after one week in hibernation; and, after three weeks, there is an inactivation of both the parietal and the chief cells and an absence of zymogen granules within the chief cells. The large, rounded parietal cells do not give a typical response when stained with hematoxylin and triosin as they appear vacuolated and contain very fine cytoplasmic granules. The smaller chief cells also have a vacuolated appearance. After three months of hibernation, neither the parietal nor the chief cells respond to staining with hematoxylin and triosin.

In this instance, however, twenty-four hours after awakening the animal from hibernation, the parietal and chief cells are again apparent with the reduction of the contained mucus noted previously.

While these changes are taking place within the stomach, the mitotic figures in the crypts of Lieberkühn, which are a common feature of the sections of the jejunum of the warm and awake squirrels, are reduced in number after as little as one week of hibernation. By the end of three weeks a very few mitotic figures are demonstrable and, after three months in hibernation, no mitotic activity can be noted, and little further change occurs in the crypts of Lieberkühn.

Within twenty-four hours after awakening the squirrel from hibernation, mitoses are again taking place in the crypts of Lieberkühn. In warm and active squirrels, Paneth cells, which

are pyramidal in shape, contain fine purple granules when stained with Gomori's aldehyde fuchsin. They do not stain as darkly in sections taken from animals in hibernation.

No major changes are noted in the large intestine during hibernation except that the amount of mucus in the goblet cells of the epithelium is reduced during the period of hibernation from that normally noticed in warm and active animals. Twenty-four hours after the squirrel's awakening from hibernation, however, the colon shows a normal amount of mucus in the goblet cells.

We can see here that, in general, the changes in the digestive tract during hibernation are those that one would normally expect to occur in an animal which is not taking in food. The empty stomach collapses until it is possible to note its opposite walls in one single histological section. There is a progressive increase in both the secretion and storage of mucus as long as the animal remains in hibernation, and this covering of the epithelium with mucus may have the effect of preventing adhesions between the closely opposed walls of the stomach and may also serve to prevent autodigestion of the mucous membrane by residual acids or enzymes. Immediately after death, autodigestion of the mucous membrane begins and a similar pattern can be expected to develop during hibernation. Failure of such a process to take place during hibernation can most likely be charged to the mucus-containing cells of the stomach. The presence of mucus in the chief or zymogenic cells may serve to delay the diffusion of pepsin precursors or to inhibit the action of pepsin itself and thus prevent damage to the cells of the inactive mucosa. The decrease in activity and, apparently, in numbers of both parietal and chief cells would be expected with the inaction of the digestive tract and the absence of the necessity of producing the antecedents of pepsin and hydrochloric acid.

The mitoses in the crypts of Liberkühn serve to replace cells subject to normal attrition in the digestive tract. With the cessation of digestion during hibernation this is no longer a necessary activity. The Paneth cells do not respond in hibernation as in starvation, perhaps due to the lowered temperatures accompanying the condition of hibernation.

The submaxillary gland of the Arctic ground squirrel is similar to that of the rat, with a separate lobe for its mucous and serous portions. When sections of the submaxillary are treated by the periodic acid Schiff method, a heavy concentration of

PAS-positive granules appears in the distal parts of the cells adjacent to the lumen of the well defined ducts (Pl. 1, fig. 2). In sections stained with hematoxylin and eosin, the intralobar ducts from a warm and active squirrel show columnar, striated cells with centrally placed nuclei surrounding the definite lumina of the ducts.

The intralobar ducts of animals in hibernation demonstrate inactivity. There is a progressive loss of PAS-positive granules in the cytoplasm of the ductule cells in the process of hibernation. This loss is observable as early as one week after entering hibernation and continuing diminution of the granules is demonstrable after about three weeks of hibernation, when only isolated granules are observed in the cells treated by the periodic acid Schiff reagent (Pl. 1, fig. 3). After three months of hibernation, there is no evidence of granules in these cells.

The general configuration of the cells likewise changes with time during hibernation. A hematoxylin and eosin stained section of the submaxillary from an animal in hibernation for three months demonstrates a decrease in the size of the cells with an apparent "piling up" of the nuclei. The lumina of the ducts appear narrowed with many being collapsed.

Within twenty-four hours after awakening from hibernation, the PAS-positive granules can be noted. They are first seen at the basal part of the cell and migrate toward the distal part. In sections stained with hematoxylin and eosin, the cells again appear columnar in shape and have centrally placed nuclei. The lumina are also again well defined.

The acinar part of the submaxillary of the warm and active squirrel contains pyramidal cells with basally located nuclei when the serous part of the gland is stained with hematoxylin and eosin. The cytoplasm shows a finely granular appearance (Pl. 1, fig. 4). With the onset of hibernation, there is a gradual collapsing of the acini, and, in a section stained with hematoxylin and eosin and taken from a squirrel in hibernation for three months, the acini appear smaller and give a disorganized appearance. The nuclei are centrally placed with only a small amount of cytoplasm surrounding them. The cytoplasm has lost much of its granular appearance (Pl. 1, fig. 5). However, twenty-four hours after awakening from hibernation, there is a reactivation of the acini which again appear pyramidal and contain basally placed nuclei. The cytoplasm resumes its normally granular appearance.

The atrophy of the exocrine pancreas during hibernation was observed first by Carlier (1893) who noticed the characteristics of the islands of Langerhans. Bierry and Kollmann (1928) as well as Vendrely and Kayser (1951) utilized Brachet's technique which reveals the ribonucleic acid in these cells. In our studies, the appearance of the exocrine pancreas of a warm and awake squirrel, when stained with Gomori's aldehyde fuchsin, shows pyramidal cells resting on a delicate reticular membrane and converging toward a central lumen. These cells possess centrally located nuclei and a granular cytoplasm (Pl. 1, fig. 6). The exocrine pancreas from an animal in hibernation for three months shows acini so densely packed with coarse positively-staining granules as to mask the appearance of the nuclei (Pl. 2, fig. 1). Kayser (1957), in citing the work of Genest, points out that, after twenty-four hours of hypothermia, the cells of the exocrine pancreas of the white rat are filled with secretory concretions and there is apparently some resemblance between the appearance of the exocrine pancreas of the hypothermic rat and that of the hibernating ground squirrel, although the time elements involved are greatly different. As early as twenty-four hours after awakening from hibernation the granular accumulation has disappeared from the acini.

This behavior is essentially what one would expect in the glands of the digestive system during hibernation when no food is either swallowed or digested. The rapid recovery of these glands might be taken as an adaptation to the necessity of the recently awakened animals' taking food and being able to swallow and digest it. It has been suggested that the collapse of the glands during hibernation might be indicative of the fact that protein material is being withdrawn from these inactive cells for use in essential body metabolism.

The work on the effects of hibernation on teeth has been fragmentary indeed. Sarnat and Hook in 1942 utilized the thirteen-lined ground squirrel and concluded that all stages of tooth development were retarded by hibernation. A closer investigation of this phenomenon in the Arctic ground squirrel indicates the following changes.

In the normal situation within the warm and active animal, the incisal dentin is a wide, homogeneously calcified, outer layer which extends from the surface of the tooth to the uncalcified predentin (Pl. 2, fig. 2).

In contrast, the process of hibernation is accompanied by a deterioration of bone and teeth. There is an indication of a

disturbed calcification process in the region of the incisal dentin after an animal has been in hibernation for a period of three weeks. Here, contrasted to the more recently formed, disturbed dentin, there is still present a thin outer zone of homogeneously calcified dentin (Pl. 2, fig. 3).

In addition to the effects on dentinogenesis, the alveolar bone also undergoes similar degenerative changes during hibernation. The interradicular and interseptal bone of the molariform teeth show a high degree of calcification in the warm and active animals (Pl. 2, fig. 4). This can be contrasted to the condition in an animal in hibernation for three months for, in the hibernating animal, the progressive loss of both interradicular and interseptal bone is obvious (Pl. 2, fig. 5).

Examination of skulls of squirrels kept in captivity for extended periods of time shows a marked development of caries rather consistently in the molar and premolar teeth. Field-caught animals do not show such a predilection for caries, and it is believed that caries are not related to hibernation but more likely to conditions of captivity, including diet.

Investigation of the pattern of caries shows all stages of involvement from early lesions to the fracturing of the coronal part of the tooth. More advanced carious lesions are actually characterized by the invasion of the dentinal tubules.

Pulp involvement is not an uncommon occurrence. Invasion of pulpal areas to produce degenerative changes often occurs and the disintegration of the pulp may extend into the root to produce an apical abscess. The pulpal inflammation likewise may result in a hollowing out of the tooth canal and, in addition, the epithelial attachment may proliferate deeply along the sides of the root.

In addition to the problem of caries and the effects on bone and dentinogenesis, animals in hibernation for from three weeks to three months exhibit various degrees of periodontal involvement. Osteoporosis of the alveolar bone has been demonstrated in animals which have been in hibernation for three months.

In an animal in hibernation for three weeks, the interproximal region of the two premolars shows a loss of trabeculation, although much less than that observed in some animals which have been in hibernation for a longer period of time. However, there may be pocket formation on both the mesial and distal surfaces of two adjacent teeth. The breakdown of surface epithelium with the presence of calculus on the cementum and a loss of interseptal bone has also been noted. The downgrowth

of the epithelial attachment may proliferate into the radicular area and may be accompanied by a disorganization of the attachment fibers and a lowering of the alveolar bone. A further progression of this effect involves periodontal pockets in the mesial and distal surfaces with such extensive bifurcation involvement that no bone remains in the interradicular region (Pl. 2, fig. 6). In addition, apical abscesses may be observed with inflammatory invasion of the surrounding connective tissues. It is obvious that hibernation produces changes in the bone, periodontum, and the process of dentinogenesis, and that these changes become more marked the longer the animal remains in hibernation.

Other participants in this symposium are charged with dealing with endocrines during hibernation and with the brown fat problem, neither of which I will attempt to mention here.

The relationship of the lipids and glycogen in selected ground squirrel tissues has been the subject of much research. Kayser (in press) has distinguished two nutritional behaviors during hibernation as exemplified by (1) hamsters which often awaken in the course of their hibernation and feed, but do not become excessively fat prior to entering hibernation, and (2) those hibernators, such as the ground squirrel, which store a great deal of fat in the autumn and have less frequent periods of awakening. While this is not a hard and fast rule because squirrels have been noticed by myself, and also by Wade as early as 1930, to take food during their periods of awakening, it is sufficient for a simple division. In the hibernators of this second group, the energy is provided apparently by lipids. As early as 1857, Valentin showed that 99.3 per cent of the stored lipids were consumed during a five month hibernation. Valentin divided the organs into two types: those whose weight loss during hibernation was proportionally greater, and those whose weight loss was proportionally smaller than the weight loss of the whole animal. Thus, he indicated that the main function of certain organs was to constitute energetic storages whereas other organs wore out to a certain extent during fasting.

Investigations of the liver, tongue muscle and heart of hibernating and non-hibernating squirrels indicate a reduction of liver and muscle glycogen in hibernators below that of the warm and awake animal. The reverse situation, however, is true for heart muscle. Lipids were noted to be in greater concentration in the livers of hibernating squirrels than in the livers of non-hibernators. The problem of glycogen synthesis during hiberna-

tion has not yet been solved. Forssberg and Sarajas (1955), in experiments with carbon-14 labeled glucose, could not resolve this question which was raised as early as 1849 by Regnault and Reiset.

Coupled with the unsolved problem of glycogen synthesis is the fact that the cold environment itself may qualitatively alter lipid metabolism in hibernators as Fawcett and Lyman (1954) proved. The iodine number of the stored fat rose in a hamster kept at cold temperatures, but that of a rat left in a cold environment did not particularly change.

In addition to glycogen synthesis, modern histochemical techniques have made it possible to detect concentrations of lipids and alpha amino acids in tissues as well. Utilizing the ninhydrin Schiff reaction as described by Lillie in 1954, it was possible to demonstrate the content of alpha amino acids in liver and tongue muscle of hibernating Arctic ground squirrels. In hibernating squirrels, there is a marked reduction in the amount of demonstrable alpha amino acid. In the livers of warm and active squirrels, however, there is, as one would expect, an abundance of alpha amino acids from normal protein hydrolysis. A similar picture is presented by tongue muscle of hibernators and warm and active animals in which the protein degradation is quite readily demonstrated during the active stages.

The absence of appreciable alpha amino acids in the livers of hibernating squirrels indicates a low rate of protein metabolism during hibernation. We know from previous work that digestion has ceased and no alpha amino acids are coming from the digestive tract. This, however, does not mean that there is no hydrolysis of protein occurring. It is possible that protein degradation is taking place at such a low rate as to have the resultant products used almost as rapidly as they are produced and thus leaving none to accumulate in the liver of the hibernating squirrel. There is also the possibility that the reduction and perhaps cessation of protein metabolism may simply be due to the lessened body temperature of the hibernator. At lowered body temperatures it is possible to postulate that the reactions incident to protein catabolism may not be able to proceed. Haurowitz (1950) has mentioned a reduction in the rate of denaturation of protein solutions by storage at low temperatures, perhaps indicative of reduction of other types of protein metabolism as well at lower temperatures.

The muscle alpha amino acid content likewise follows the pattern of that of the liver in showing negligible protein degradation in the muscle cells of hibernators while the presence of the alpha amino acids in the muscle cells of the warm and awake squirrels is indicative of a greater protein catabolism in those animals.

Tissue lipids were investigated using frozen sectioned tissues stained with Sudan black B (Lillie, 1954). The lipid content of the livers of those animals which were warm and awake was negligible. The livers of the animals in hibernation, however, showed definite fat droplets in the cytoplasm of the parenchymal cells. One animal, supercooled to −4.6°C in hibernation, showed an even more dramatic concentration of fat in the liver with the fatty degeneration of the organ quite pronounced. On staining with hematoxylin and triosin, large intra- and extracellular fatlike vacuoles were observed. In the sections stained with Sudan black B, large fat globules were present in and around the individual liver cells.

While the liver is not normally mentioned as a fat depot in warm and active animals, this condition apparently changes with the onset of hibernation. The livers of the active animals showed a negative sudanophilia, while the livers of squirrels in hibernation showed the presence of fat droplets. With the exception of the supercooled animal mentioned previously, the liver lipids and glycogen are present in inverse proportion one to another. The lessened liver glycogen and the presence of lipids strongly suggest the metabolism of lipids to account for the energy of awakening from hibernation.

It is apparent that the process of hibernation places great demands upon the tissues of the body. All the tissue resources are directed toward the problem of maintaining the animal's metabolism at the minimal level necessary for life. This involves disruption of glands and a cessation of all activity not immediately germane to the process of living at the lowest possible metabolic level. While such structures as the digestive tract and its related glands appear not to suffer from this process and recover relatively rapidly, the effects on certain other structures such as teeth may be deleterious. The mobilization of materials from individual tissue cells to be utilized elsewhere undoubtedly stresses the tissues concerned, as indicated by the eosinopenia and leucopenia observed by authors investigating the circulatory changes during hibernation (Lyman *et al.*, 1957; Villalobos *et al.*, 1958).

The hibernator apparently balances on a very narrow line between maintenance of life at such a level that recovery is possible and maintenance at such a level as will eventually lead to death. From the evidence of the tissues, the process of hibernation seems to be a precarious method of survival at best and one from which many animals do not awaken.

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DISCUSSION FOLLOWING MAYER'S PAPER

LYMAN asked if, in the case of an animal hibernating for three weeks at a time, MAYER knew whether or not this was continuous hibernation for three weeks. MAYER replied that it was continuous hibernation, since animals were attached to a permanently recording device. LYMAN then addressed KAYSER and asked if his record for continuous hibernation was 130 days, as he (LYMAN) understood. KAYSER replied, "Lately I have again become interested in the problem of the durations of the phases of uninterrupted hibernation, particularly in contrasting hibernation of mammals to hibernation of poikilotherms. I think — but I have no evidence — that hibernation of poikilotherms is characterized by the absence of any discontinuity. This seems to be the general rule. In hibernating mammals, hibernation *must* be interrupted. Thus, they occupy an intermediate

position between poikilotherms and artificially cooled homoiotherms. In the latter the duration of hypothermia may not exceed 24 hours; in poikilotherms it lasts for several months; in hibernators there are interruptions — every fourth day, on the average, in the European hamster (*Cricetus cricetus*), a "bad" hibernator, and about every 21st day in the European ground squirrel (*Citellus citellus*), a "good" hibernator.

"With this thought in mind, I made precise measurements during the winters of 1957-58 and 1958-59. The above data are the results; they have not been published separately, but appear in my manuscript on hibernation sent in February, 1959, to Pergamon, Inc., publishers.

"In 1952 (Arch. Sci. Physiol., **6**:193) I presented curves of the whole hibernation of ground squirrels (*Citellus citellus*). In these curves the longest uninterrupted sleeps lasted 18 and 21 days, respectively. My experiments of 1957–58 and 1958–59 showed phases of 21 days to be "normal" in ground squirrels in November-December.

"In 1940, I gave an example of periodic hibernation prolonged for nearly a year in the common dormouse (*Glis glis*). I said, 'We have kept a dormouse without food or drink, sheltered from noises and tactile stimulations, from March till the end of July. The animal slept for weeks and months, without interruption . . .'

"At that time, I did not want to determine the duration of uninterrupted sleep, but to show that by keeping the external conditions constant (temperature $+5.0° \pm 0.5°C$), it is *possible* to have a hibernator hibernating for nearly a year, and that the endocrines of the hibernator remain in winter condition. I saw no arousal from April 1st, 1938 (a day on which the thermostat failed to work correctly) till the end. It is thus possible that the animal did not awake for more than 90 days. I cannot affirm it with certainty, having no longer any formal document on this point. My attention was called to the fact that I had made an "abnormal" observation by Lyman and Chatfield (Physiol. Rev., **35**:403, 1955, p. 413). This is why I tried later to determine the durations of uninterrupted hibernation. I saw then that in refrigerators regulated at $5 \pm 2.0°C$ this duration was 21 days for the ground squirrel. No dormice were available (100-150 gm), so I could not repeat the same experiments in this species. In the garden dormouse (*Eliomys quercinus*), weighing 30-60 gm, I found that the arousals may be incomplete:

the animal increases its temperature, but does not awake entirely. The actograph (statograph with a tambour and a recording tambour) showed no activity in spite of the increase of the superficial body temperature. For this reason I think that continuous recording of the microclimate by thermistors in contact with the animal would be the best method of monitoring the phases of uninterrupted hibernation. Actography, as I used it with small-sized animals, may lead to inexact conclusions. The longest continuous hibernation I have seen in the hamster is 8-9 days."

FISHER remarked that it would be good to know not only the extremes, but the average of such a function. LYMAN said the average varied with the species. KAYSER said the average for the hamster was about 4 days, for ground squirrels in November, 20 days, and in December, 25-27 days. FISHER indicated that when such figures are given one must be careful to specify the temperature of the environment.

LYMAN then asked MAYER what ambient temperature was used in his animal experiments. MAYER replied that they had animals hibernating at different temperatures, but their standard for low temperature was 4.2°C.

FOLK then referred again to KAYSER'S remarks, indicating that he was about to give figures for continuous hibernation similar to KAYSER'S, with a maximum of 26 days in one animal. STRUMWASSER asked FOLK for his criteria of continuous hibernation. FOLK replied that they had observed animals three times a day (first winter) and twice a day (2nd winter) in considerable detail, using a marking technique and counting respirations, and that he was positive that animals had not come out of hibernation. STRUMWASSER then remarked that convictions, based on visual observation, that animals continuously hibernate for long periods of time are quite subjective; actographic recording of the animal's activities is at least objective, but still subject to a great deal of criticism when involved in determining the length of maintained natural hypothermia. FOLK said he was convinced actograph recordings were not necessarily more sensitive than counting respirations.

SMITH asked MAYER if there had been any histological studies made during natural arousal, and whether one would expect partial changes to occur. MAYER said he would expect

changes to occur in proportion to the time involved, but no one yet has sacrificed animals at short intervals during the process of arousal from hibernation to show this in detail.

WIMSATT asked if the animals in MAYER'S experiments were fed prior to sacrifice. MAYER replied that they were not fed between time of arousal and sacrifice, although they were allowed water.

DAWE asked if MAYER holds to a point of view that the animal hibernates "all over," that all tissues change. MAYER replied that in his opinion every tissue participates in hibernation, that changes in hibernation are not simply changes in an endocrine or two, but a change implicating all tissue. LYMAN inquired specifically as to the changes in the heart during hibernation. MAYER said that glycogen was the only thing he had studied in the heart. He did notice that myocardial glycogen increases during hibernation. LYMAN agreed that this is what he had found also.

KAYSER asked if MAYER knew of any specific cause for the deterioration of teeth in hibernation. MAYER said the submaxillary gland is not operative, hence no protection is afforded from that area. However, a majority of the caries seen seem not to be directly attributable to hibernation. The development of caries is more a dietary problem. People in dental schools have trouble getting animals to develop caries, but these animals do it automatically.

MENAKER asked where the thermistor used in the experiments was located. MAYER said it was not next to the animal, but that most of the thermistors ended up lying on the floor of the burrow. The temperature in the nest where the animal is would be higher because of the insulating value of the nest. He said that when he indicated that none of the burrow temperatures went below 16°F, the temperature at the animal wasn't anywhere near this low.

SOUTH mentioned a recent paper on caries which showed that carious conditions correlate directly with involution of the thyroid (hypothyroidism) since such involution results in a change in the buffering capacity of the salivary gland secretions. In this case, caries do not correlate with dietary factors directly, but with thyroid activity which, in turn, might be correlated

with pre-hibernation involution of the gland. SOUTH also remarked that, with respect to MAYER'S figures indicating the lowering of body temperature, the difference between that temperature and the environmental temperature would require (according to a rapid calculation he had just done) the evaporation of too much total body water over a 24-hour period to make it a probable explanation; in other words, such an explanation would require a heat pump, which is highly unlikely. Granted the accuracy of the recordings, such results may be caused by the lag which may exist in the methods of measurement. MAYER replied that he did not postulate a heat pump but felt that the evaporative losses at the higher environmental temperatures at which the animal hibernated, together with the low relative humidity, would give the effect described.

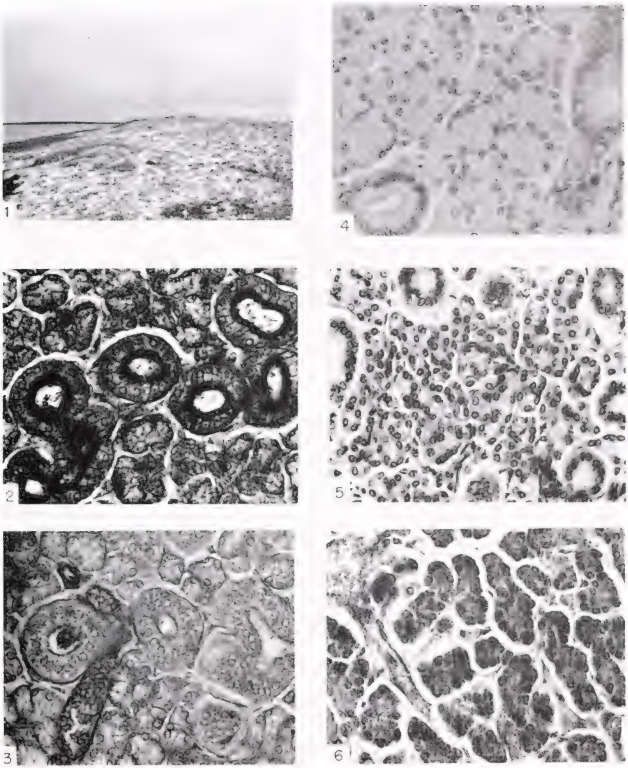

Plate I

Fig. 1. Typical ground squirrel burrow site on Arctic slope of Alaska. Near Meade River, Alaska, September, 1952. Fig. 2. Section of the intralobar duct of the submaxillary gland from a warm and active Arctic ground squirrel. Heavy concentrations of PAS-positive granules surround the lumina. Fig. 3. The intralobar ducts of the submaxillary gland of an Arctic ground squirrel in hibernation for three weeks. Only isolated PAS-positive granules can be seen. Fig. 4. The acinar part of the submaxillary gland of the Arctic ground squirrel stained with hematoxylin and eosin. Finely granular cytoplasm, pyramidal cells and basally located nuclei are typical of the warm and awake squirrel. Fig. 5. The acinar part of the submaxillary gland of the Arctic ground squirrel stained with hematoxylin and eosin. The small, disorganized acini with centrally placed nuclei and non-granular cytoplasm are characteristic of the ground squirrel in hibernation for three months. Fig. 6. Pancreatic acini of the Arctic ground squirrel stained with Gomori's aldehyde fuchsin. Finely granular cytoplasm is characteristic of the non-hibernating squirrel.

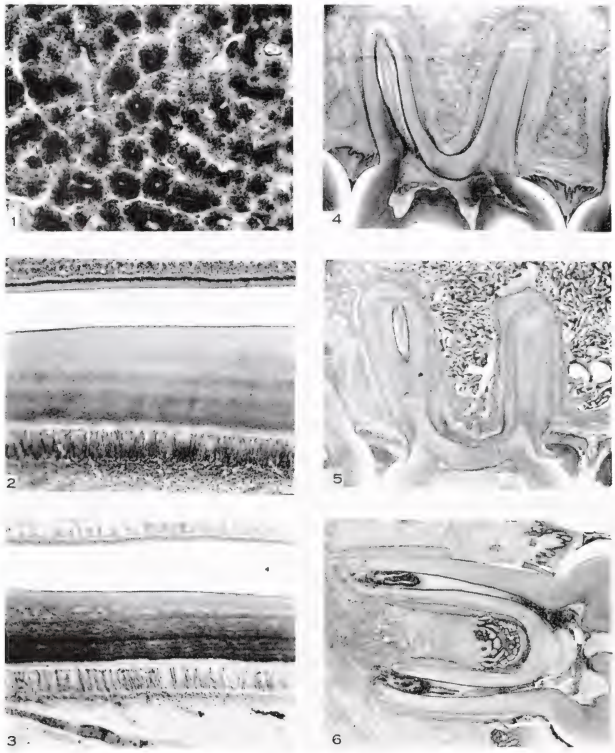

Plate 2

Fig. 1. Pancreatic acini of the Arctic ground squirrel stained with Gomori's aldehyde fuchsin. Coarse granules mask the cellular nuclei and are characteristic of the animal in hibernation. Fig. 2. Homogeneous calcified dentin of the incisor of a warm and active Arctic ground squirrel. Fig. 3. Incisal dentin from a ground squirrel in hibernation for three weeks. Deficient dentin is characterized by an increased number of interglobular spaces. Fig. 4. Upper molar from a warm and active Arctic ground squirrel. The interseptal and interradicular bone is compact. Fig. 5. Upper molar from a squirrel in hibernation for three months. Osteoporosis of the spongiosa of both interseptal and interradicular bone is obvious. Calculus is present in the interproximal gingivae. Fig. 6. Extensive carious involvement of the upper first molar of a squirrel in hibernation for three months. Obvious bifurcation involvement with the loss of attachment fibers and bone. Periapical abscess present.

VIII

SEASONAL VARIATIONS IN PHYSIOLOGIC FUNCTIONS OF ARCTIC GROUND SQUIRRELS AND BLACK BEARS[1,2]

By Raymond J. Hock
Arctic Aeromedical Laboratory
Ladd Air Force Base
Alaska

Hibernation is a cyclic phenomenon, exhibited annually except in the case of the bats (Eisentraut, 1933; Hock, 1951). It appears that, due to the shortened season into which hibernators must compress their annual cycle of activity, nearly all facets of their life history and consequently their physiologic functions reflect the fact that they are constantly preparing for hibernation, hibernating, or recovering from that condition. Thus, the whole year is involved in the cyclic phenomenon that is hibernation.

Let me define hibernation as "a periodic phenomenon in which body temperature falls to a low level, approximating ambient, and heart rate, metabolic rate, and other physiologic functions fall to correspondingly minimal levels." The Arctic ground squirrel fits this definition as a hibernator well, and is in fact as deep a hibernator as any of the more intensively studied European or temperate American species.

On the other hand, the black bear is not a hibernator by this definition, and elsewhere I have proposed the term *carnivorean lethargy* to describe the condition found in bears and presumably some other carnivores (Hock, 1958).

Annual Cycles

The Arctic ground squirrel, *Citellus undulatus,* is the hibernator found farthest north in North America, as it extends nearly to Point Barrow in Alaska. Due to the short season in which it can remain active in Alaska, this species has greatly compressed its annual sequence of activities. In my study area in the

[1] The contents of this manuscript reflect the personal views of the author and are not to be construed as a statement of official Air Force policy.

[2] The animal experimentation was conducted according to the "Rules Regarding Animal Care" as established by the American Medical Association.

Talkeetna Mountains near Anchorage, the male squirrels emerge rather precisely beginning April 21, despite variation in the seasons. Figure 1 shows a summary of five years of observation. This figure also shows that the time of entrance into hibernation is nearly as precise, for the later dates shown here reflect the fact that I was present in the area a little later each year.

GROUND SQUIRREL ENTRANCE & EMERGENCE DATES

SPRING		FALL
	1950	
NO OBSERVATIONS.		LAST DATE APPR. 2 OCTOBER COLD, LIGHT SNOW 1 OCTOBER "NORMAL SEASON"
	1951	
FIRST DATE, APPR. 22 APRIL. 3' SNOW, BARE SPOTS "NORMAL" SEASON		LAST DATE 5 OCTOBER COLD, HEAVY SNOW 18 & 5 OCTOBER EARLY SEASON
	1952	
FIRST DATE 21 APRIL. 4½ – 6' SNOW, NO BARE GROUND. 3 WEEKS LATE SEASON.		LAST DATE 7 OCTOBER WARM (+10°C.), RAINY LATE SEASON.
	1953	
FIRST DATE 21 APRIL 2 – 4' SNOW, ½ BARE GROUND. 2 WEEKS EARLY SEASON.		LAST DATE 9 OCTOBER WARM, CLEAR. 1ST SNOW 18 OCTOBER LATE SEASON.
	1954	
FIRST DATE 21 APRIL. 2½ – 4' SNOW, PART BARE. 3 – 4 WEEKS EARLY SEASON.		LAST DATE 12 OCTOBER COOL, LIGHT SNOW "NORMAL" SEASON
	1955	
NONE SEEN UNTIL 27 APRIL. HEAVY BLIZZARD ON 16 APRIL. 5½ – 8' OF SNOW. LATE SEASON.		NO OBSERVATIONS

Fig. 1. Dates of first and last seasonal appearance of Arctic ground squirrels near Anchorage, Alaska, in 1950-55, with notes on weather, ground cover and seasonal comparisons. (Reprinted from Cold Injury. Trans. 5th Conf. Josiah Macy Found., 1958.)

Figure 2 shows the events that comprise the seasonal cycle of the Arctic ground squirrel. On May 1, the females begin to emerge and breeding begins almost at once. By about May 10-15, all females are pregnant, and the gestation period is 25 days (Mayer and Roche, 1954), so that the young are born in late May to early June. The young stay in the nest about six weeks, and begin to emerge about July 5-10. The mother cares for the young for another 10 days to two weeks, after which they are left to care for themselves. At the end of this period of care (early August), all the population rapidly acquires sufficient fat reserves for hibernation. The males have already started increasing in weight in early July. By about September 10-15, the

number of squirrels is noticeably reduced, and it appears that the females have started to hibernate. The adult males are active until about the end of September to October 5, and after that date I have found only young of the year active. Normal last date of activity is about October 12, although a few aberrantly immature animals may still be found after this.

Thus the season of activity is about 138 days for the females and 168 days for the males.

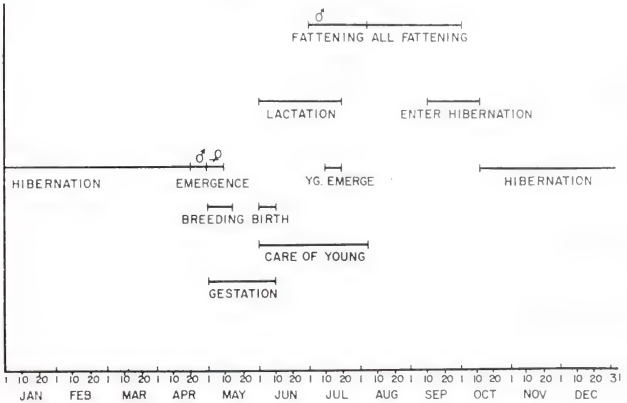

Fig. 2. Average dates for the various events of the seasonal activity cycle of Arctic ground squirrels near Anchorage, Alaska.

The black bear, in Alaska, emerges from its winter quarters about mid-April to early May, perhaps due to melt-water from the snow running into the dens. At this time, there is no available food, and, as a matter of fact, the bears seem not to be hungry or very thirsty. Bears eat mostly grass in summer, with an occasional tidbit of mouse, ground squirrel, or salmon. They also dig for roots, and in the fall eat the abundant berries of many kinds, especially *Vaccinium* spp. The bears are active until middle or late October, and it appears to be the first heavy snow of winter that sends them to the dens.

Breeding occurs in the latter half of June, and the young are born about mid-January. However, the gestation period is not precise, as one of my females had young this year about January 25, and dates from December to March may be found in the

literature (see especially Baker, 1904). The variation is probably due to the discontinuous development of this species (Hamlett, 1935). The young are born in the winter dens and nursed by the female on her body resources. The cubs (normally 2) weigh 250-400 grams each at birth, and on emergence 3 months later about 4 to 5 kilograms each.

Weight Variation

It is generally agreed that the acquisition of adequate fat reserves is a necessary preparation for hibernation in most species that hibernate (Lyman and Chatfield, 1955). The loss of weight throughout the period of hibernation has been studied

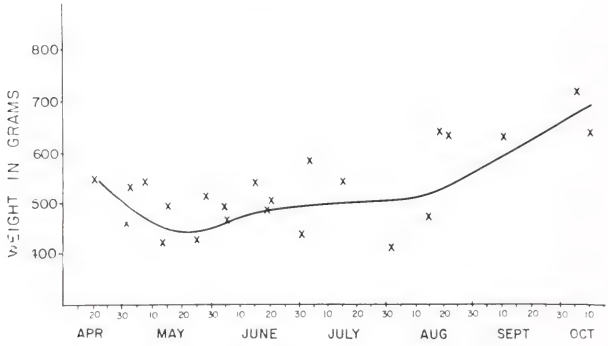

Fig. 3. Average weights of Arctic ground squirrels for each date. A five-year period is represented without distinction as to years. Data from near Anchorage, Alaska.

by several authors, but the weight cycle during the active period has been neglected.

Male adult Arctic ground squirrels emerge in April with weights averaging about 550 gm. There is no food available at this time and, in fact, snow 120 to 240 cm in depth may lie uniformly over the ground. In some years, there are patches of bare ground, and some dead plant debris may be utilized. Small amounts of food are stored in the burrows in fall (Mayer, 1953; Krog, 1954), and may be used at this time. However, there is a weight loss at this season, and lowest weight is reached in mid-May (Fig. 3). Weight of the entire adult population then

remains relatively stable until late July, with the males slowly gaining weight while the females lose due to lactation and care of the young. Beginning in the first half of August, the entire population rapidly fattens until the onset of hibernation. Females reach weights of about 600 gm before disappearance, while males go to 700 gm or above. Males disappear in early October, apparently when weights approach 750 gm. I have rarely caught one over this weight, and maximum weight recorded in this area is 960 gm.

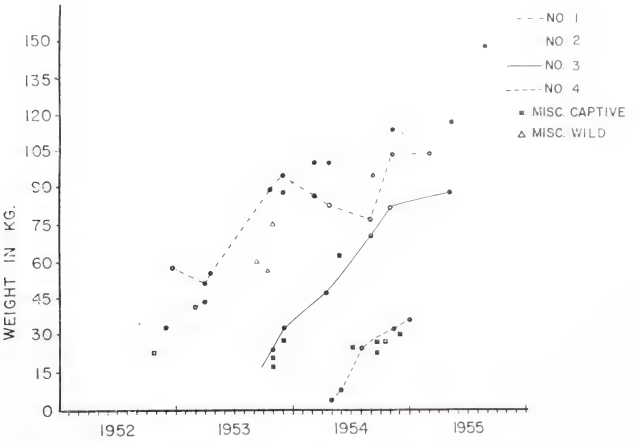

Fig. 4. Weights of black bears plotted against time of year.

Weight loss during hibernation in captivity is normal for hibernators, so I will not discuss it.

The black bear is perhaps more interesting from the point of view of weight loss, for the inability to radically drop its metabolic rate as do the true hibernators imposes a more severe energy demand on the bear during its stay of nearly six months in the winter den. Figure 4 shows change in weight of two bears over a period of nearly 3 years. It will be seen that there is a loss of about 9 kg during the winter denning season in 1953-54 for bear 1. During the following summer we attempted to feed the bears as they would find available calories in the wild, and weight loss continued. About August 1, they were

provided with all they could eat (12,000 calories/day) and they gained up to 25 kg in two months. It appears that this is not unlike the weight cycle seen in the wild, for on September 1 I have seen bears with over 5 cm of subcutaneous fat and great masses of depot fat. Dr. Robert Rausch of the Arctic Health Research Center has provided data on a four-year old male brown bear which he collected on Kodiak Island on September 10, 1952. Total weight was 195 kg, and fat weighing 20 kg was removed. It was estimated that total fat weighed about 30 to 32 kg, or that 16 per cent of the weight was fat. Subcutaneous fat varied in thickness from 40 to 53 mm over the hind quarters.

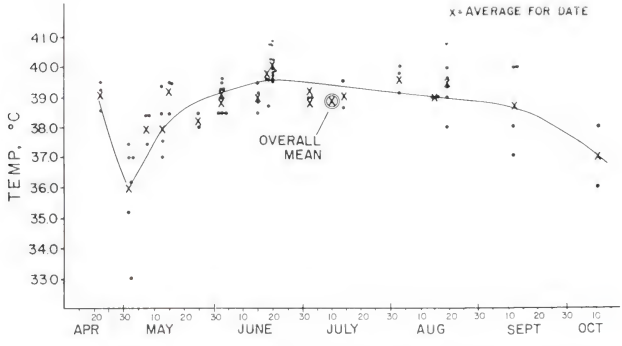

Fig. 5. Rectal temperatures of wild Arctic ground squirrels plotted against date. Five years are represented.

It appears that wild black bears may lose up to 20 to 30 kg during the winter denning period. Assuming the average adult weight to be 150 kg, this would be a loss of about 15-20 per cent of their weight. Ground squirrels lose nearly 30 per cent, although their torpor is more profound. This difference in percentage weight loss is probably due to the great difference in total body mass of the bear and ground squirrel, and is in itself tribute to the efficiency of deep hibernation.

Body Temperature Variation

It has long been stated that hibernators have a greater lability of temperature while active than that found in homeotherms (Johnson, 1931; Gelineo, 1938). However, in the course of other

studies I discerned a seasonal variation in rectal temperature in active squirrels. Figure 5 shows this variation in wild squirrels plotted against date. There is no apparent relation to ambient temperature, sex, or degree of fatness. Figure 6 shows averages of rectal temperatures of about 100 captive squirrels kept at 10°C, approximately the average temperature of the wild squirrel's habitat. There is a progressive fall in temperature under

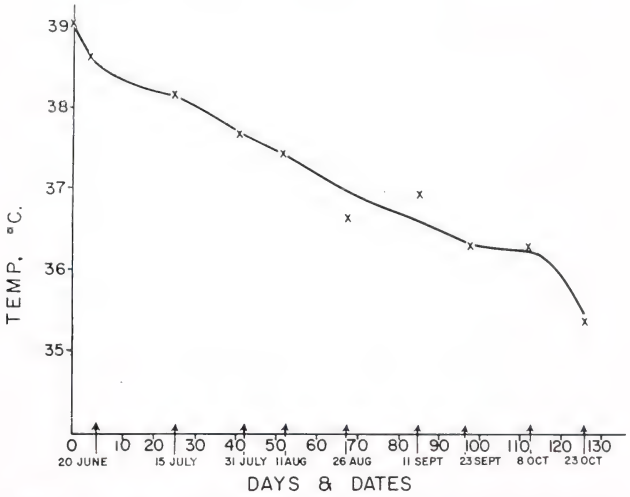

Fig. 6. Average rectal temperatures of captive Arctic ground squirrels plotted against date.

these captive conditions which is not discernible in the wild squirrels until just prior to the onset of hibernation. This points up the fact that data on captive animals is not necessarily valid for extension to the wild or natural state. Therefore, it appears that wild ground squirrels exhibit four different phases of temperature control during the year, as follows: (1) the low temperatures found during hibernation; (2) the variable temperatures found for a short period after emergence; (3) high and relatively constant temperatures during most of the active season; (4) falling temperatures just prior to entrance into hibernation.

During hibernation, temperatures close to ambient are recorded. Normally, I have kept my animal quarters at 4° ±2°C,

and have had highly successful hibernation at these temperatures. One winter an ambient temperature of 10°C was maintained with slightly less success.

It has been stated that hibernating Arctic ground squirrels must endure temperatures below 0°C, as they inhabit permafrost regions. However, they avoid the low-lying ground, and burrow in river banks and other high sandy or gravelly locations where permafrost is well below the burrow level. Certainly, on exposure to 0°C the normal reaction is one of arousal from hibernation, although I have in one case reduced rectal temperature to below

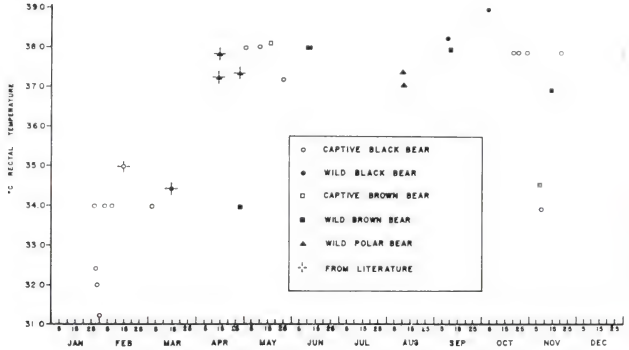

Fig. 7. Rectal temperatures of bears plotted against date. (Reprinted from Cold Injury, Trans. 5th Conf. Josiah Macy Found., 1958.)

0° by exposure of a hibernating squirrel to −20°C. The squirrel recovered, with extensive freezing damage. Other squirrels in the same situation aroused from hibernation or did not arouse successfully (Hock, 1958).

The black bear is able to maintain high constant temperatures while active in any ambient temperature. Actually, when I first measured rectal temperatures in the winter dens, I found to my surprise that the only recorded bear temperatures were some taken on the Arctic expeditions of the 1820's, and 30's on polar bears. I have since been able to record a number of active bear temperatures, of black (*Ursus americanus*), brown or grizzly (*Ursus arctos*, cf. Rausch, 1953), and polar bears (*Thalarctos maritimus*). Most are close to 38°C, as seen in Figure 7. Polar

bears seem to be about one degree lower, and perhaps they are more variable in rectal temperature characteristics.

In contrast, all temperatures taken on lethargic bears are around 34°C, as seen in Figure 7. On one occasion, a rectal thermocouple stayed in place for three days, and recorded a temperature of 31.2°C (Hock, 1957). This is the lowest recorded normal temperature for bears.

Dr. Rausch has recently been able to record the rectal temperature of a wild black bear denning near Anchorage, Alaska, on February 16, 1959. Air temperature was −16.5°C, and immediately after it was shot, the bear's rectal temperature was 33°C. This point is not shown in Figure 7, but it will be seen that it is in the range of the captive black bear readings.

Metabolic Rate Variation

It appears obvious that the rationale for the evolution of hibernation is for purposes of conservation of energy so that the animal's food supply, either in the form of depot fat or physically stored food, can be made to support it for the required period. The metabolic rate reduction during hibernation has been admirably treated by several authors, notably Kayser (1940, 1957). I should like to treat another aspect, namely seasonal variation of metabolic rate of active ground squirrels. Hart (1957) has recently reviewed such seasonal variation, but no hibernators were included in his review.

Twelve ground squirrels that had not hibernated in winter were used for the determination of metabolic rate under ambient temperatures of 30° to −10°C. Figure 8 shows the average oxygen consumption of these animals. A second and larger group was studied in summer under the same scheme. Both groups were kept at 10°C, ambient temperature, and the principal difference between them was that the winter group had been captive longer and was under a constant photoperiod, while the newly captive summer group was under a decreasing photoperiod. Figure 8 shows the average values for the latter group also.

It is first evident that Erikson's (1956) results on the Arctic ground squirrel were not duplicated in this study, as he found no essential change in metabolic rate from 10° to 50°C. Sullivan and Mullen (1954) found no difference between 5° and 25°C, in this species. Figure 8 shows a well-marked relationship of metabolic rate to ambient temperature. Kayser (1957) has

pointed out that Erikson's failure to find dependence of metabolic rate on ambient temperature is not in line with studies on other hibernators in the active condition.

It is also apparent that the ground squirrel does not show the same lack of seasonal variation in metabolic rate as reported by Irving *et al.* (1955) for the red squirrel. Butterworth (1958) has stated that the Arctic ground squirrel has two annual molts, although other northern ground squirrels have only one. This may explain the seasonal variation here shown, as Irving *et al.*

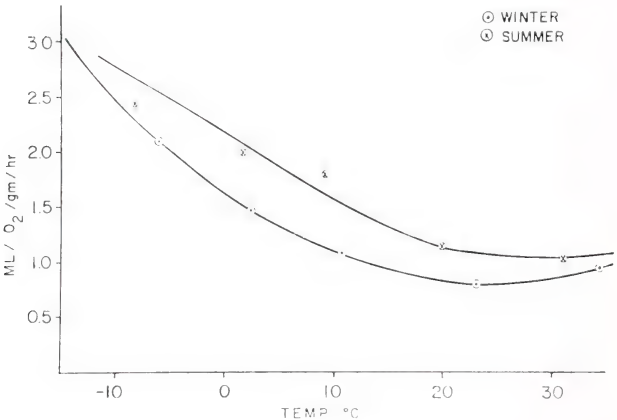

Fig. 8. Average metabolic rate of Arctic ground squirrels in relation to ambient temperature. Winter and summer curves are shown.

(1955) concluded that the seasonal variation shown by the red fox was due to better insulation of the winter pelage over the summer pelage, while that of the red squirrel might be due to little pelage insulation change. However, it appears that this reduction in metabolism of the winter group may rather be a reflection of: (a) lower body temperature, and (b) considerably reduced general activity. It may also be that endocrine factors are concerned, but I have no direct evidence on this point.

Metabolic rate during hibernation at 6° ±2°C ranges from 0.03 to 0.10 ml O_2/gm/hr, with an average of about 0.063 ml/gm/hr, which is clearly within the expected range. It thus

appears that the Arctic ground squirrel exhibits no metabolic or temperature peculiarities due to its hibernation in far northern areas underlain by permafrost.

Figure 9 shows the metabolic rate of an active yearling black bear in relation to varying ambient temperature. The normal seasonal variation of metabolic rate is seen, similar to that shown by Irving *et al.* (1955) for well-insulated Arctic animals. Figure 10 shows the metabolic rate of active bears in winter compared with that of a lethargic bear, plotted over 24-hour periods.

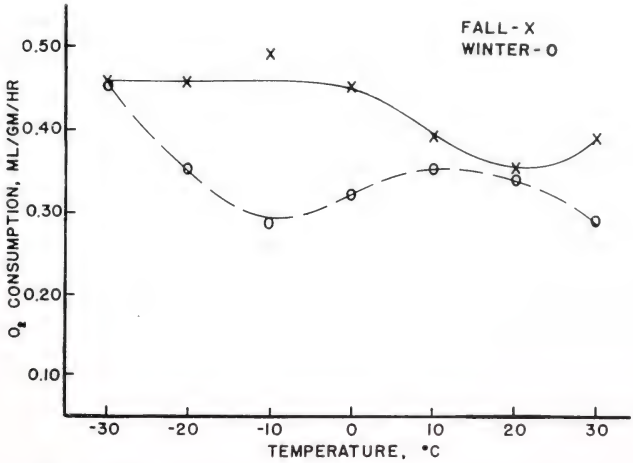

Fig. 9. Metabolic rate of active yearling black bear in relation to ambient temperature. Early fall and late winter curves are shown. (Reprinted from Cold Injury. Trans. 5th Conf. Josiah Macy Found., 1958.)

These animals were housed in adjacent cages, and their dens were metabolic chambers. It can be seen that the metabolic rate in lethargy, where reduction in rectal temperature is only of the order of 7°C, is about 50-60 per cent of normal. This is greater than that evident in man under hypothermic anaesthesia (Virtue, 1955), so the bear apparently is superior to man or dog in this respect. I must emphasize that I was unable to determine rectal temperature during this study, and the fall in den temperature, found when outdoor temperature fell to −40°C and below, may

indicate that the bear's temperature fell lower than the 31°C given above. However, previous calculations on weight loss in winter indicated that the energy expenditure during lethargy must fall to about ½ to ⅔ of normal levels when rectal temperature was assumed to fall no lower than 31°C. It should also be

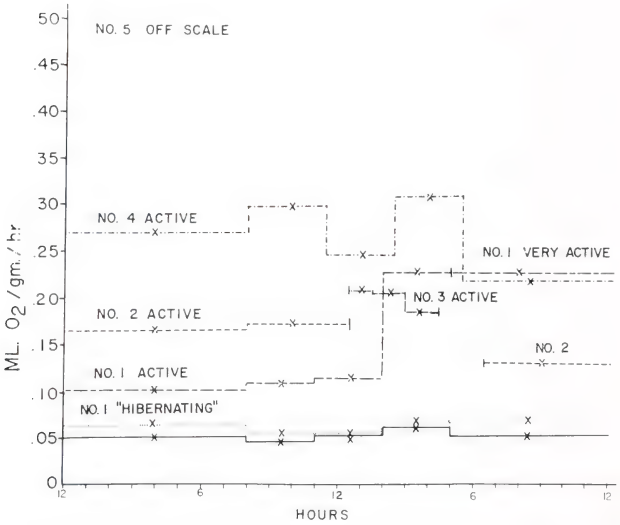

Fig. 10. Approximate 24-hour metabolic rates of black bears in the active and "hibernating," or lethargic condition in winter. Values are provisional only, but are useful for comparative purposes.

emphasized that the data in Figure 10 are rough in nature, and have not yet been properly analyzed. However, for comparative purposes, they are adequate to demonstrate the reduction in metabolic rate found under the lethargic state assumed by the black bear.

Breeding Cycle

It appears that hibernators have made several breeding adaptations to the shortened season they have left available to them after emergence from hibernation (Hock, 1958). In this short

season, they must not only carry out the breeding function, but must allow the young animals adequate time for the acquisition of fat reserves sufficient for the approaching period of hibernation. My contention that all seasonal activities of hibernators are geared to the fact that they do hibernate is perhaps most clearly demonstrated by this curtailed reproductive period.

The Arctic ground squirrel breeds almost immediately after the emergence of the females, about May 1-10. In order for this to happen, gametogenesis must occur, at least in part, during hibernation. It is apparent that it does so, for the males emerging on April 21 and for a few days thereafter have spermatids present in the seminiferous tubules, and the testes are enlarged and descending, though not yet scrotal. By May 1, the testes are scrotal, and the lumina of the epididymis and seminiferous tubules are packed with motile spermatozoa.

It is apparent that this is one of the reasons, at least, why the males emerge earlier from hibernation than do the females. The females begin to emerge on May 1, and have ovarian follicles ready to rupture. Breeding occurs almost at once, and ends about May 10-15. Ovulation in ground squirrels occurs only when copulation has occurred. The testes soon become smaller and retract to the inguinal position, so that no late breeding is possible. Spermatogonia and some primary spermatocytes are the only elements present in the seminiferous tubules during summer.

In the fall, shortly before the onset of hibernation, the testes begin to grow in size and secondary spermatocytes begin to appear. The situation in the female is more difficult to determine, perhaps due to their earlier entrance into hibernation.

During the winter growth continues, as evidenced in the male largely by the increasing size of the testes and the large number of spermatocytes. It appears that the spermatids are first evident only just before the emergence from hibernation. The growth of the ovarian follicles continues slowly through the winter. Lyman and Chatfield (1955) have postulated that this growth of gonads occurs during the periodic arousals, rather than during the actual periods of hibernation. I have no information to shed light on this point and, in considering the preparation for a short season, it is of little significance whether the growth occurs at high or low body temperature.

Summary

It appears evident that hibernation is a cyclic phenomenon, both in its occurrence and in the preparation for it. In short, the whole year's activities are related to the fact that the animal will hibernate for a considerable time. Certainly this is true of the Arctic ground squirrel for, due to the short season imposed on it by its high northern distribution, it behooves this mammal to compress its annual cycle of breeding and the acquisition of adequate nutritive reserves into a very short period. To this end its whole cycle of activity is geared, and many physiologic functions have responded by showing seasonal cycles of various intensities during the active season as well as during hibernation.

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DISCUSSION FOLLOWING HOCK'S PAPER

MAYER noted that the body temperature of 36.4°C which he had cited previously was on captive squirrels which are used to being handled; he believed this temperature substantiated the data HOCK presented. HOCK noted that his (HOCK'S) "average" wild value was 38.8°C.

MUSACCHIA asked what evidence was available as to the periodicity of arousals in the wild of Arctic ground squirrels during the hibernating season because there is an apparent lack of experimental data and, therefore, no significant evidence concerning the nature of arousal, either periodic or sporadic, during the hibernation of the Arctic ground squirrel. HOCK said there is none, but he is currently analyzing data on this subject on captive animals accumulated over a nine-year period. For this purpose he notes the periods of activity, but he does not claim this method is precise.

PENGELLEY inquired as to how one can be sure the animals arouse every two or three weeks. HOCK said he could not be sure, but this was the longest normal period for this species. MAYER remarked that he could substantiate this particular periodicity from his laboratory observations, and that he could substantiate the fact that animals aroused in the wild during the winter.

PENGELLEY then asked if they eat when they become active in this way. HOCK said they do store food and, if food is available, they will eat. PENGELLEY said *Citellus lateralis* may or may not eat or drink during an arousal, but certainly they do not need to. HOCK said that in the captive squirrels he did not provide them with water, but with lettuce, and that no water would seem to be available to wild hibernating squirrels. MUSACCHIA added that if these animals eat at the time of their arousal, they must clean mucus out of the alimentary canal so that an initial or limited feeding may be of little nutritional benefit. MAYER referred to his own experiments, noting that for studying histological changes he did not provide food. Animals without food simply arouse from hibernation and stretch or move around before re-entering hibernation. If animals come out of hibernation and run around, this active state may last for several days. MAYER further pointed out that after 24 hours of such activity, the metabolism of the animal is running at "full blast." HOCK cited the example of an animal which had never been seen in the active state over more than a three-month period, but it turned out that although it had not been seen active in this period, nevertheless it had aroused since it had disturbed the activity indicator several times.

WIMSATT asked if HOCK had calculated the maximum time an animal could remain active during the hibernating season without prejudicing its survival through the season until spring.

HOCK replied that he did not know exactly, but if one takes the hibernating metabolic rate at $\frac{1}{30}$ of the active rate, then an animal which has enough food stored to hibernate 180 days can survive six days of activity fasting at the same environmental temperature with the same weight loss.

MORRISON remarked that the figure given on fat deposits in the bear seemed very low. He (MORRISON) had data on one denned bear in which the adipose tissue was 40 per cent of the body weight. He indicated that his figures showed that if the bear had gone on without a reduction in metabolism and body temperature, it could have lasted 120 days. MORRISON stated that in this bear the temperature (heart) was in the normal range. He asked if it might not be possible to obtain low rectal temperatures in a bear with a normal deep body temperature due to the dense fecal plug that closes the rectum during hibernation. HOCK replied that he made deep rectal insertions which gave him figures of 38.0° ±1.0°C for active bears and a low of 31.0°C for bears in the "hibernating" or lethargic state.

PROSSER asked if there were any seasonal figures available on rectal temperatures of polar bears — for those in water and out of water. HOCK replied that he had taken one rectal measurement of a polar bear that had been shot in the water and the body recovered. The temperature drop observed was small, although a more labile body temperature is indicated in this species. He had also taken the rectal temperature of a young polar bear he had outside his laboratory just before he came to this meeting: the T_R was 37° to 38°C. The latter is a maximum for this species.

IX

OBSERVATIONS ON A COLONY OF CAPTIVE GROUND SQUIRRELS THROUGHOUT THE YEAR[1]

By Barbara R. Landau
Department of Physiology
St. Louis University School of Medicine
St. Louis, Missouri

and

Albert R. Dawe
Office of Naval Research
Chicago, Illinois

Some general aspects of hibernation and manifestations of a torpid condition have been considered in several species, including some in the natural (wild) state. For purposes of intensive investigation, however, it is desirable to have these animals in captivity. Bringing them into the laboratory may affect their behavior and activity, but it permits better control and closer observation. People have been observing hibernators for several centuries, but their studies usually involved one or two animals rather than a colony.

One who is interested in hibernation is interested in how and why it occurs. Throughout the years attempts to identify the factors controlling hibernation have followed either of two general approaches. One of these has dealt with characteristics that are unique to hibernating species, and which enable, or cause, these animals to shift from the homeothermic to a seemingly poikilothermic state. It is well known that the tendency to hibernate is a cyclic phenomenon, associated with the season of the year. It is also well known that there are other phenomena which show seasonal cycles in most hibernating species, such as body weight, endocrine gland function and sexual activity. Numerous attempts have been made to relate the occurrence of hibernation directly to one or more of these seasonal cyclic phenomena.

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The other approach to hibernation is concerned with environmental conditions which favor, or are necessary for, entrance into the hibernating state. Among these conditions, cold and temperature, confined air and carbon dioxide, and food or lack of it, have been frequently mentioned, as well as light, sound and mechanical disturbances. A consideration of the hibernating state must take into account both seasonal cyclic activity and the factors in the environment, because the effectiveness of either in inducing hibernation will be modified by the other. The material to be presented is based upon observations of the behavior and responses of a colony of ground squirrels, and at times of individual animals. The data were collected from April, when the first animals were caught, throughout the year until the following summer. There were daily observations of the behavior of the colony in regard to certain cyclic phenomena, and in response to the environment. The effect of specific alterations in the environment were also determined.

The colony. The colony consisted of 109 ground squirrels (*Citellus tridecemlineatus*). Of these, 21 were caught in April and early May (spring caught), 63 in mid-June (summer caught), and 25 in late September and early October (fall caught). The ground squirrels caught in spring and summer were housed in pairs. In the fall all animals were transferred to individual cages in the general animal quarters, where they were kept until they were placed in the cold. The temperature of this room ranged between 20° and 29°C; hence it was designated the "warm room."

To encourage hibernation, animals were placed in a "cold room." The temperature was not held constant, but was allowed to fluctuate somewhat with that outside. It never fell below -1°C, and it exceeded 12°C twice before April 1. Individual hibernacula were provided, which consisted of an insulated metal box, with a detachable screened lid. Each box contained cotton waste for nesting. An attempt was made to have food and water available at all times for those animals in the cold, even though hibernation is said to be more likely if the animals have been deprived of food and water (Johnson, 1930; Kayser, 1950). It was felt that, because of additional disturbances, these animals would be aroused more frequently than in nature, and would probably need the extra energy supply. During the summer, and while in the warm room, the animals were fed and watered on alternate days. The diet consisted chiefly of sunflower seeds.

Accommodations in the cold room were limited, and since these animals were being used in another series of experiments, the same animals were not in the cold room all winter. Individuals were taken to the laboratory, sometimes for several days, and often were aroused from hibernation. Every ground squirrel was in the cold room part of the winter.

From late November through April the ground squirrels in the cold room were checked daily (except for 2 days), usually between 8 and 10 a.m. The warm room animals were checked daily at first, and later on alternate days. Checking the colony consisted of noting: (a) room temperature, (b) barometric pressure, and (c) condition of each animal, as to health and state of activity.

Body weight. Among the cyclic phenomena which were observed was body weight. It has long been asserted that the weight of the animal contributes to the tendency to hibernate (Horvath, 1881; Wade, 1930). Johnson (1930) reported that heavy animals (146-185 gms) hibernated 62 per cent of the time in the cold, while light animals (86-125 gms) hibernated only 39 per cent of the time in the cold. Johnson's animals, however, weighed considerably less than those studied here, for after midsummer, all of our animals weighed more than 100 gms, and some exceeded 250 gms.

The animals were weighed when caught, and at the beginning of many experiments. Among the spring and summer caught animals, males were heavier than females, but by mid-summer this difference had disappeared. Young ground squirrels gained weight rapidly, and by September they had caught up with the fattening adults. Animals caught in the fall weighed approximately the same in October as the spring caught animals had weighed in April. They gained weight slowly after capture, but remained lighter than animals caught earlier. The spring and summer caught animals, as groups, hibernated 28.6 and 23.4 per cent of the time in the cold, while the lighter weight fall caught animals hibernated 41.4 per cent of the time in the cold. So, in general, heavy animals hibernated more than light ones, but as shown in Figure 1, high percentages of time in hibernation were attained at much lower weights among the fall caught animals. Thus, size is not an absolute criterion for hibernation.

At least two factors could have contributed to this difference between catches. Many spring and summer caught animals were not placed in the cold room until late in the season, when hibernation was less likely to occur, whereas more fall caught animals

were placed in the cold room early in the winter. Secondly, a diet of sunflower seeds, which the summer caught animals received for a longer period of time, may simply result in obesity, without enhancing biochemical preparation for hibernation.

Gonads and sexual activity. The endocrine glands of most hibernating species go through an annual cycle, returning to a relatively dormant state before the hibernating season (Kayser, 1950, 1953; Foster, Foster and Meyer, 1939). This involution

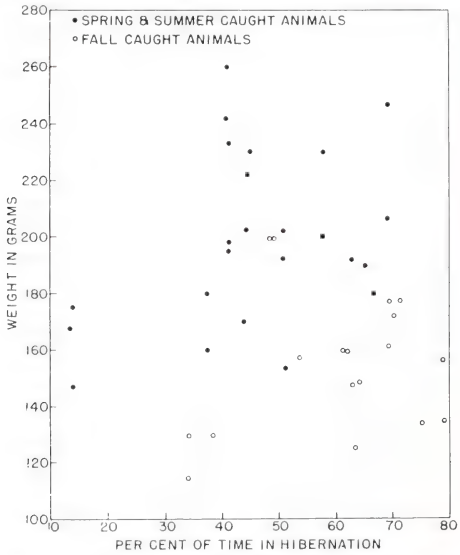

Fig. 1. Relationship between body weight and the percentage of time that an animal hibernated when kept in the cold room.

is generally considered to be associated with the time of year, rather than with hibernation, since it occurs prior to the hibernating season. For example, the testes of male ground squirrels are scrotal only during the few brief weeks of the mating season in the spring. However, by mid-December most of the males in the warm room showed some degree of scrotal enlargement. Four of these animals were transferred to the cold room, and 3 hibernated after 21, 33 and 52 days in the cold, respectively. It

was determined at autopsy that the testes of these animals had become abdominal. The fourth ground squirrel did not hibernate, and the testes were still scrotal when it was sacrificed after 62 days in the cold. By early March there were also signs of scrotal enlargement in several males in the cold room. Johnson and Wade (1931) have also observed scrotal testes in January and February among ground squirrels kept in the laboratory.

It is said that ground squirrels will not live together except during the mating season (Johnson, 1917). However, our spring caught animals were housed in pairs (male and female) from mid-April to as late as mid-September without incident. In view of the signs of gonadal descent in January in warm room males, attempts were made to encourage mating in two different pairs. There was some excitement when the animals were first placed together, but no fighting, and both pairs lived together peaceably for a month or more during the winter.

General activity. The daily check of the colony included a rating of the voluntary activity of each animal at the time of the check, according to an arbitrary scale, as follows:

"1" — Very active. Running about, excited.
"2" — Active. Out of the nest, eating or moving about.
"3" — Alert. In the nest, but awake.
"4" — Asleep, or nearly so. Drowsy, eyes closed.
"5" — Borderline. Sleeping soundly, not awakened by checking. Breathing slowed, but body not cool to the touch. Also includes animals in the process of arousing from hibernation.
"6" — Hibernating. Characteristic position, respiration very slow or not apparent. Body cool to the touch.

Although this activity rating was subjective, it provided an indication of the activity of the ground squirrels that were not hibernating. The results of this tabulation are shown in Figure 2. All animals were relatively inactive in late autumn and early winter. Cold room animals were considerably less active than those in the warm room. After mid-March the cold room animals became increasingly active. This coincided with the scrotal enlargement noted in the cold room males. It is of interest that, unlike non-hibernating species, ground squirrels in the cold room tended to be less active than those in the warm room. This parallels the lack of endocrine response to cold, shown by Deane and Lyman (1954), and supports the concept that cold is not stressful to these animals.

Several processes are known to diminish measurably as the hibernating season approaches, such as metabolism (Kayser, 1953) and body temperature (Dubois, 1896; Benedict and Lee, 1938), as well as the endocrine involution previously mentioned. Heart rate and breathing rate are functions that are related to metabolism, and they also show seasonal variation. Table 1 contains data for resting heart and breathing rates obtained from unanesthetized ground squirrels. In spite of a rather small sampling, the resting rates tended to decline progressively from relatively high levels in April. Some of the animals in the

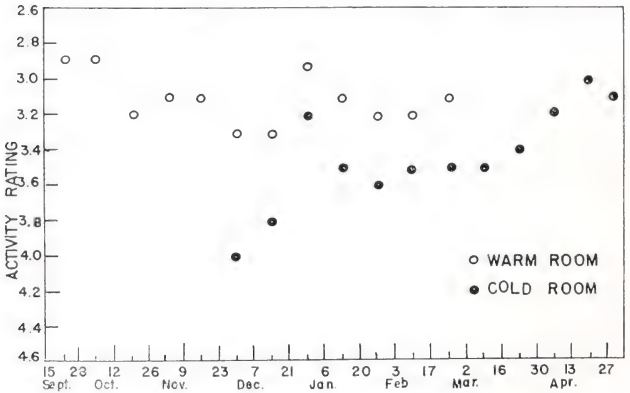

Fig. 2. General activity of all animals that were not hibernating. Animals were rated according to an arbitrary scale (see text), in which smaller numbers indicate greater activity.

spring exhibited very rapid rates, suggesting that the values given might not be true resting rates. However, it is quite possible that the animals were more excitable at this time. The sluggishness in autumn is probably one facet of the broad overall preparation for seasonal hibernation.

Depth of hibernation. In the course of experiments on animals in hibernation, it became apparent that arousal was more easily triggered in some animals than in others. For example, if an animal had lost one of the electrodes (implanted previously for other experiments), a wound clip could sometimes be applied without initiating arousal, yet at other times merely bringing

a box containing an animal into the laboratory would set off the arousal process. On one occasion, thermocouples and electrodes were imbedded subcutaneously in 9 hibernating ground squirrels. All of the animals were aroused by this procedure, but their reactions differed markedly. Some began to squirm immediately and continued to do so, while others stirred only when the skin was pierced, and then but slightly. An hour later the greatly disturbed animals were quite alert, while the others had burrowed down into the nesting in typical hibernating position, although their breathing was rapid. It was felt that these ground squirrels must have been in different depths of hibernation, and hence they were said to be in "shallow" and "deep" hibernation, respectively.

TABLE 1

Heart and Breathing Rates (Range and Average) of Quiet Unanesthetized Ground Squirrels at Different Times of the Year

Date	Number of animals	Resting Rate per Minute Heart	Breathing
April 16-30	12	284(188-444)	180(126-255)
May 1-15	12	277(180-456)	169(108-276)
May 16-31	7	256(240-276)	207(136-348)
June 16-30	7	223(168-246)	169(108-276)
September 28-October 20	5	212(130-260)	
January 17-February 8	6	190(120-260)	81(45-113)

Following this observation, the response of hibernating animals to the handling necessary for recording experiments was noted whenever possible, and the animals were classified as "shallow" or "deep." Of the 46 occasions in which this was done, 19 ground squirrels were considered "deep" and 27 were judged "shallow." "Deep" animals were found to be in the first to eleventh day of consecutive hibernation, an average of 4.9 days. "Shallow" animals had been in hibernation for from 1 to 5 days, an average of 2.0 days. It seemed that by the fifth day of hibernation, an animal was likely to be "deep," so 5- or 6-day ani-

mals were used for experiments whenever possible. In this way fairly good success was obtained in connecting an animal for recording without initiating arousal. Later in the season, after about the first of March, 1- and 2-day animals occasionally were found to be "deep." It appeared that in the early part of the season a longer time was required to reach "deep" hibernation.

The only previous references to depth of hibernation have been those (Horvath, 1881; Wade, 1930; Johnson, 1931) concerning "partial hibernation," at relatively high environmental temperatures, and the observation of Lyman and Chatfield

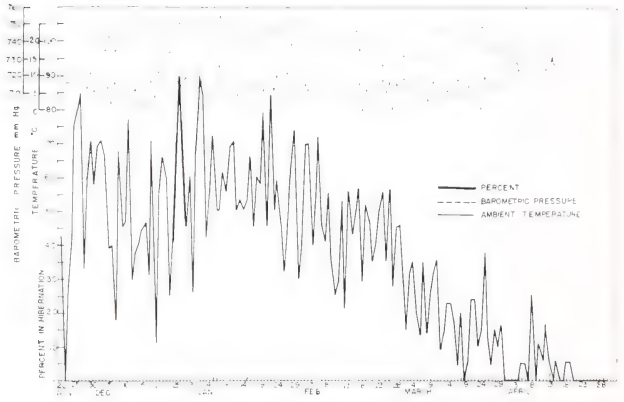

Fig. 3. Daily tabulation of the percentage of the colony hibernating in the cold room, with barometric pressure and ambient temperature.

(1955) that hibernating hamsters may be more sensitive to externally applied stimuli on one day than on the next. These data suggest that the depth of hibernation does vary, and that several factors may be involved. Among these factors are: (1) The number of days in consecutive hibernation, since animals which had been hibernating for 5 or 6 days were more likely to be in what was termed "deep" hibernation. (2) The number of entrances into hibernation, or the season itself, for late in the hibernating season animals were found to be "deep"

after a shorter period. This may in some way be related to the phenomenon of successively lower test drops, described by Strumwasser (1959a). (3) The depth of hibernation is probably also a species-related characteristic, for Lyman and Chatfield (1955) noted that various species differed in their sensitivity to externally applied stimuli while hibernating. Marmots and hedgehogs are relatively insensitive to stimulation, while hamsters are notoriously easy to arouse.

The depth of hibernation does not seem to be cyclic in quite the same sense as the phenomena previously discussed, but it does modify the effect of environmental conditions upon the tendency to hibernate, and it may be cyclic.

The tendency of an animal to hibernate is modified by environmental conditions which do, or do not, favor the hibernating state. We were able to observe the effect of a number of these factors, both incidentally and experimentally. Information gathered in the daily check of the cold room animals provided much of the data, which is summarized in Figure 3. The percentage of the colony in hibernation fluctuated widely, but declined from about February 1. Ambient temperature and barometric pressure varied randomly with no noticeable change until April 1, when temperature tended to rise.

Ambient temperature. Figure 4 relates the percentage of the colony hibernating on a given day to the ambient temperature. Because of a decreasing tendency to hibernate after about the first of February, data from early and late winter have been handled separately. In early winter an ambient temperature of between 5° and 10°C was most favorable for hibernation, but in late winter a lower temperature (0-2.5°C) was optimal, a range of 0-10°C for the entire hibernating season. High temperatures (12.5°C) were more apt to arouse animals than temperatures near zero, even in early winter. The ground squirrels apparently had a different range of response to cold in early and late winter, for they required a lower temperature as winter progressed.

Large daily fluctuations in percentage of the colony hibernating led to the suspicion that perhaps sudden temperature changes might be partly responsible. When the change in environmental temperature from that of the previous day was plotted against

the percentage of the colony in hibernation, there was little correlation, due to the wide scatter.

In late autumn and well into the winter, animals in the warm room hibernated briefly. Perhaps the term "partial hibernation" is desirable, because the ambient temperature was at least 20°C, which automatically precluded the extremely low body temperatures ordinarily attained. Torpor and body cooling were observed

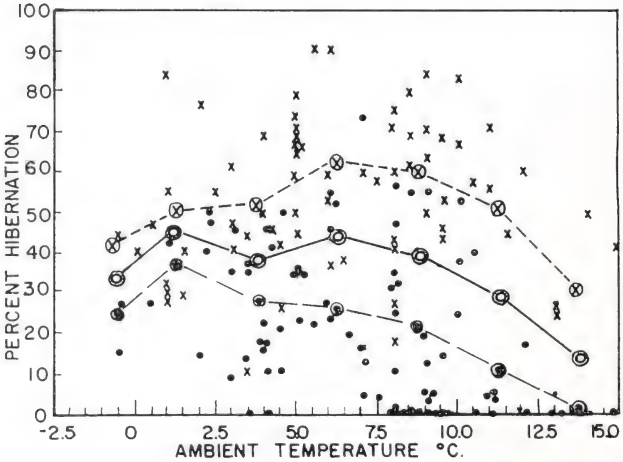

Fig. 4. Relationship between ambient temperature and percentage of the colony hibernating. Crosses are values obtained from 21 Nov. - 30 Jan. Closed circles are values obtained from 1 Feb. - 30 April. Short-dashed lines with crosses are averages for 21 Nov. - 30 Jan. Long-dashed lines with closed circles are averages for 1 Feb. - 30 April. Solid lines with open circles are averages for the entire winter, 21 Nov. - 30 April.

41 times in 18 different animals in the warm room between mid-September and early February. There were undoubtedly other occurrences, since part of this time the warm room animals were checked on alternate days.

In this colony, hibernation was seen to occur at temperatures ranging from 0°C in the cold room to a maximum in excess of 20°C in the warm room. This is essentially in agreement with

the work of others, on this and other species. The ground squirrels hibernated most when the ambient temperature was between 5° and 10°C.

Barometric pressure. Barometric pressure, like temperature, showed no obvious relationship to the percentage of the colony in hibernation (Fig. 3), but Figure 5 shows that the higher pressures favored hibernation, especially in late winter. The

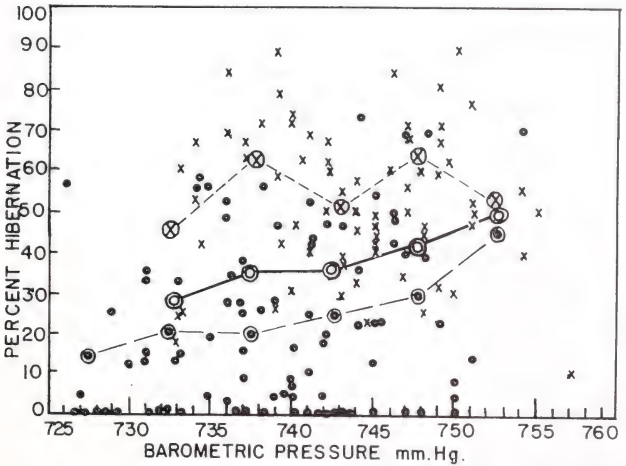

Fig. 5. Relationship between barometric pressure and percentage of the colony hibernating. Symbols as in Figure 4.

optimum atmospheric pressure was above 750 mm Hg. Day to day fluctuations were great, tending to mask any effect of daily pressure variations, but if such a relationship existed, it was with a slightly rising pressure. This is supported by Lindemann (1951) who found a relation between the onset of hibernation and a rising barometric pressure. The effect of pressure, however, was less apparent than that of temperature.

Several hibernating animals were placed in a specially constructed cold chamber, where they were subjected to pressures ranging from +32 to −60 mm Hg for from 5 minutes to 5 hours, followed by sudden return to atmosphere. Arousal was not initiated in any of these animals.

Season. Season usually is considered a factor contributing to the hibernating state, mainly as winter versus summer. It has already been noted that the tendency to hibernate diminished as the winter progressed. Figure 6 shows the hibernation trends of the animals in the cold, as well as the temperature and pressure, averaged by 2-week intervals. In all but the spring caught group, the percentage of the animals hibernating began to decline about February 1, although ambient temperature did not rise until April 1. Thus the tendency to arouse was not a simple reflection of elevated environmental temperature. The spring

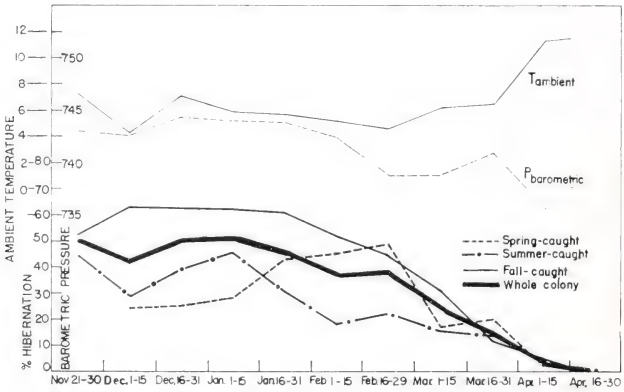

Fig. 6. Two-week averages of the percentage of the colony in hibernation throughout the hibernating season, shown by groups and as a whole.

caught animals hibernated very little until they had been in the cold for about 6 weeks, and thus hibernated very little until quite late in the season. Animals kept in the warm room until late in the winter showed little tendency to hibernate when they finally were put in the cold, but those animals which had been hibernating well all winter also hibernated less in the spring. This is best seen among the fall caught animals, which as a group, were in the cold longest and hibernated most. Ground squirrels placed in a cold chamber (5°C) in June and July frequently hibernated after several days, and lethargy was observed in the warm room from August throughout the fall. This leads to the conclusion that, although natural hibernation

may occur throughout most of the year, the tendency to hibernate is greatest from November to February.

Externally applied stimuli. Rotating an animal in its nest on the turntable of a record player was used as a motion stimulus. Speed of rotation was varied by changing the inertia of the turntable, and ranged from 7 to 70 rpm. Stimulation lasted for from 5 to 15 minutes. Acceleration was gradual, but deceleration was usually brought about abruptly by hand. All rotation was in the horizontal plane, but with the animal's head curled under, it was not possible to tell which semicircular canals had received primary stimulation. In 28 trials arousal was never initiated, either by acceleration or abrupt deceleration.

It is often assumed that a satisfactory hibernaculum must be quiet but this has not been adequately substantiated. It might be mentioned, however, that the cold room used in this study was not particularly quiet, but the noise level was fairly constant. For auditory stimulation, a hibernating animal in its box was put in a small refrigerator (with the door open), which was placed squarely in front of a loud-speaker in a sound-proof room. Sound of any intensity and frequency within a wide range could be delivered through the speaker from an adjoining control room. Experimentally, 7 animals were subjected to the auditory stimulation, applied for 2 minutes every 10 minutes. The stimulus consisted of various combinations of frequency and intensity as well as random noise. One animal showed absolutely no response to the successive two-minute periods of sound and noise, even at maximal intensities. Several attempts to duplicate the experiment resulted in aroused animals. These arousals were at least partially due to the difficulty in maintaining a cold environment, as it was necessary to leave the refrigerator door open during auditory stimulation. It is of interest that the animal that was not disturbed by sound had been hibernating for 7 consecutive days, whereas 5 of the 6 that were aroused had been in hibernation for 1 to 4 days.

On several occasions it was necessary to leave a light shining through the transparent cover of the experimental cold chamber, directly on a hibernating animal. This never initiated arousal, and at least one animal is known to have entered the hibernating state while the light was on. Aroused animals in the light tend to seek out dark corners of the nest, and bury themselves under the nesting. By doing this, and curling up as they do, it is not likely that light could exert a very strong effect.

This does not preclude a possible effect of light upon the tendency toward seasonal hibernation. The animals in the cold room in this study were kept in darkness 24 hours a day, which would resemble the condition in a sealed burrow during the winter.

There were several indications that a hibernating animal has an elevated pain threshold. It has been mentioned that wound clips could occasionally be applied without initiating arousal. Intraperitoneal injections of cold solutions of physiological saline, epinephrine and atropine have been given without causing arousal. This was not the rule, however, for such procedures usually initiated arousal.

The inconstant results that have been obtained upon stimulation of sensory receptors of animals in hibernation can no doubt be explained at least partially on the basis of the concept of differences in the depth of hibernation, i.e. "shallow" and "deep." Mild stimulation readily initiates arousal in "shallow" animals, whereas "deep" animals show but a slight response of short duration. The relative insensitivity of "deep" animals to stimulation could be due to inability of the peripheral nerves to conduct, or to depression of the ascending reticular activating system. Excised tibial nerves of hamsters function at temperatures as low as an average of 3.4°C (Chatfield *et al.*, 1948). The auditory nerves of hamsters probably do not conduct below 18°C, and they are said to be functionally deaf while hibernating (Lyman and Chatfield, 1955). Cortical activity has been evoked at cortical temperatures as low as 9°C in the hamster by sciatic nerve stimulation (Chatfield *et al.*, 1951), and 7°C in the woodchuck by auditory stimulation (Lyman and Chatfield, 1953).

Thus, it seems that there is variation in the ability of nerves to function in the cold, both between species and between different nerves in the same animal. These differences probably are not related only to temperature of the nerve. Since the sensitivity of an animal varies over a period of time, during which the temperature may vary but slightly, there must either be other factors governing the ability of fibers to conduct, or peripheral nerve conduction is not the whole story.

The reticular activating system, which is associated with consciousness and wakefulness, is thought to be particularly sensitive to cold. In the arousing hamster, electrical signs of activity in the reticular activating system (high frequency, low voltage deflections of the electrocorticogram) were not found until the cortical temperature had reached 29°C (Chatfield *et al.*, 1951).

More recently Strumwasser (1959b) reported spontaneous activity in *Citellus beecheyi* at cortical temperatures near 6°C, but they were reduced about 90 per cent in amplitude. It may be that these central mechanisms are more depressed after several days at low brain temperatures. It would be of interest to compare the electrical activity of "shallow" and "deep" ground squirrels with that of hamsters, since the latter are known to be very easily aroused.

It appears that animals which are capable of hibernation differ in several respects from animals which are not capable of hibernation. These differences are associated primarily with the response to cold, and involve not only survival of the whole animal at low temperatures, but also functioning of the individual tissues, such as peripheral nerves and the heart. For a triggering mechanism to initiate induction into hibernation in a species which is capable of it, at least two conditions must obtain. One of these is that the environmental temperature must be such that the animal's body temperature can fall to low enough levels to result in lethargy. The other is that the endocrine glands, which show seasonal activity cycles in most hibernating species, must be in the inactive phase. A major role has been assigned to the endocrine system by some (Kayser, 1950), but it seems more likely that the state of the endocrines, as well as the presence of a cold environment are permissive rather than causative factors. A certain degree of biochemical preparation is very likely also essential.

Environmental conditions, such as the degree of cold, atmospheric pressure, season and external stimuli, modify the occurrence of hibernation in a group of ground squirrels, but these conditions do not actually cause hibernation.

Acknowledgments

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DISCUSSION FOLLOWING LANDAU'S PAPER

FISHER asked whether the consecutive hibernation period spoken of began with an arousal period, or began with entrance into hibernation. LANDAU replied that it was timed from entrance into hibernation, and that usually by the sixth day an animal was in "deep" hibernation. In contrast, animals in March were in "deep" hibernation after only one or two days.

STRUMWASSER asked how many animals died in the cold room. LANDAU replied that very few did so. Several animals that were exposed to rather severe weather conditions died, but after the colony was moved into the cold room only 3 or 4 of about 60 animals were lost.

POPOVIC asked if, when animals died in the cold room, they died in the hibernating state. LANDAU said she believed this to be the case. HOCK then noted that such animals fit Mangili's definition of "morbid hibernators." He (HOCK) had observed that small individuals captured in the fall of the year stay in hibernation almost continuously as if they are unable to arouse. The "morbid hibernator" is a small or improperly nourished animal that "has to hibernate." HOCK also mentioned the state of the gonads in hibernation—he noted that in the wild Alaskan ground squirrel the testes are not scrotal at the time of emergence on April 21, but that spermatozoa are present on May 1, and on that date the male is ready for mating.

ZIMNY asked two questions: (1) had LANDAU ever put a female she was sure was in estrus in the cold room to see if it would hibernate; (2) had she noticed higher food consumption on the part of animals in the cold room which would not hibernate. LANDAU replied that an experiment of this type involving an estrous female had not been tried; she stated that food was kept available *ad libitum* at all times in the cold room, and that after mid-March they ate more and had to be fed every other day. They also drank more water and were more active.

SMITH said he would like to emphasize the matter of diet. He stated that several discussions had been concerned with the length of hibernation, and also with the time animals must remain in a cold room before going into hibernation. He indicated that diet could be an important factor here, especially in considering which dietary factors animals may be able to pick up in nature during the summer (pre-hibernation period) in contrast to what an animal is given in the laboratory in the pre-hibernation period.

MAYER asked if the obesity LANDAU observed might not be due to a sunflower seed diet. LANDAU said she did not think sunflower seed diet was the only cause of weight increase, but it was one factor in the relatively heavy weights of some of the animals. MAYER asked if LANDAU thought hibernation would occur on any diet, and pointed out that 800 gm animals he had caught in the field would increase their weight to 1000 gm on an *ad libitum* laboratory diet. Food had to be forcibly withheld from such an animal in order to maintain its body weight near 800 gm. LANDAU replied that she wondered at this because her lighter weight fall-caught animals hibernated on a sunflower seed diet.

PENGELLEY asked if it were not true that animals with serotal testes would not hibernate, but when testes became abdominal hibernation would take place. LANDAU replied that this apparently was true and that the one animal she maintained for a long period in the cold whose testes did not become abdominal, did not hibernate.

PENGELLEY then asked as to the exact criteria used to determine that an animal had remained in continuous hibernation for as long as 7 days. LANDAU replied that animals were checked once each day in the morning. She conceded it

was possible that animals might have gone into hibernation and aroused again during the interval between checks. She said she had no other evidence than her subjective impression, but felt strongly that the number of times she erred in not noting this were few — simply because it takes a while for the animal to perform the cycle of entrance into and arousal from hibernation. PENGELLEY noted, on the contrary, that he had observed, in keeping hibernating ground squirrels, that they will come out of hibernation and go into hibernation in a 13-hour period.

DAWE then asked LANDAU if she would describe the observations she made of three animals that died in hibernation. LANDAU stated that when these animals were autopsied, no fat deposits were to be found.

POPOVIC then noted that a hibernator hibernates in accordance with the season. When, during hibernation, the ambient temperature drops below 0°C most ground squirrels die. LANDAU remarked that lowering the external temperature sufficiently (to 0°C) results in an increase in metabolic rate in order to maintain body temperature at 3°-4°C. If an animal is compelled to do this over a long period without arousal, it may lack sufficient energy reserves for a proper arousal.

X

AESTIVATION IN THE MOHAVE GROUND SQUIRREL *CITELLUS MOHAVENSIS*

By George A. Bartholomew and Jack W. Hudson
Department of Zoology
University of California
Los Angeles, California

Physiological information on aestivation is extremely scarce. This is in part due to the fact that most physiologists live and work in areas where adaptation to seasonal cold rather than to seasonal drought represents the major adaptation of mammals to environmental stress. In desert areas aestivation offers an effective mechanism for survival during the periods when food and water are most scarce. A number of rodents have developed this ability to aestivate and offer attractive opportunities for the investigation of naturally occurring hypothermia at relatively high ambient temperatures. The Mohave ground squirrel is a diurnal desert rodent that undergoes prolonged periods of both hibernation and aestivation. It has an extremely restricted range in the Mohave Desert of California and is sympatric with the much wider ranging Antelope ground squirrel, *Citellus leucurus*, which neither hibernates nor aestivates.

Methods

The animals used in the present study were captured during the spring and summer of 1957 and 1958 in the Antelope Valley of the Mohave Desert about three miles east of Palmdale, Los Angeles County, California. They were housed individually in glass terraria in a windowless room with a twelve-hour photoperiod and were given commercial rat food, sunflower seeds and water *ad libitum*. Under these conditions the animals were extremely docile, and survival was excellent. The temperature of the animal room varied between 22 and 27°C. Field observations on seasons of activity and behavior of *C. mohavensis* were made incidental to a year-round program of study of *C. leucurus*.

Measurements of oxygen consumption were made by placing the squirrels in an air-tight two-liter container equipped with a thermocouple and ports for the introduction and removal of air. Dry air was metered through the container at a rate of

400 cc per minute and then delivered to a Beckman paramagnetic oxygen analyzer, which, used in conjunction with a recording potentiometer, gave a continuous record of oxygen consumption. The determination of oxygen consumption during torpor, entry into torpor, and arousal were obtained by putting the animal into the respirometer and placing it in a constant temperature chamber. Oxygen consumption was then recorded continuously until the animal became torpid. Some of the arousals were spontaneous, others were induced by the disturbances incidental to measurement of body temperature during torpor.

Continuous records of body temperature during arousal were obtained by inserting a vinyl-sheathed copper-constantan thermocouple through the rectum to a depth of five or six centimeters, securing the leads to the tail with adhesive tape and then attaching them to a recording potentiometer. Oral temperatures were determined manually either by thermocouple or by quick-acting mercury thermometer.

Results

Natural history and behavior. In the Antelope Valley, Mohave ground squirrels are active above ground from early March to August. They remain in their burrows throughout the rest of the year and presumably are dormant much of this time. The young are born in the early spring. In our experience they are solitary. Under natural conditions the animals are quite tame and can be readily approached; in captivity, they are extremely lethargic and spend much of their time asleep or torpid. Despite their placid behavior, captive Mohave ground squirrels are so intolerant of members of their own species that they must be housed separately.

In captivity the animals became extremely fat. They remained fat at all seasons but tended to lose some weight during the spring. From March to August, in the laboratory, the animals were active and showed no signs of dormancy. During the remainder of the year they were intermittently torpid at room temperature despite the continuous availability of food and water and despite the frequent disturbances associated with the maintenance of other experimental animals in the same room.

Oxygen consumption and body temperature during normal activity. Continuous records of oxygen consumption obtained for many hours in the present study offered favorable opportunity for the determination of standard metabolic rate. It was possible to select from many hours of recordings those intervals showing

a minimal uniform oxygen consumption. Standard weight-relative metabolism at 23 to 26°C averaged slightly more than 0.8 cc O_2/gm/hr (Table I).

TABLE I

Sex	Wt. in Grams	cc O_2/gm/hr	Air Temp. °C
-	228	1.0	26.0
♂	232	0.9	23.0
♀	270	0.8	25.0
♂	306	0.7	24.0

Standard metabolism of alert *C. mohavensis*. The figures for oxygen consumption are rates maintained for 40 to 60 or more minutes by post-absorptive animals. All the animals were extremely fat. The oxygen consumption figures shown are based on total weight and would be at least one-third larger if they were calculated on the basis of fat-free body weight.

Although the body temperature of *C. mohavensis* varies with environmental temperature and activity, it is quite uniform at room temperature in the absence of disturbance. Rectal temperatures from five different animals measured on each of four consecutive days at 8:30 a.m. during early July, a time when the animals rarely aestivate, fell between 35.2 and 36.1°C (mean, 35.75 ±0.13).

Entry into torpor. Animals placed in the respirometer at room temperature during fall and winter and left undisturbed sometimes became torpid. Since oxygen consumption was being continuously measured, it was possible to measure metabolic rate while the animals were entering torpor. Satisfactory records were obtained from five animals. Representative records are shown in Figures 1 and 2. At ambient temperatures of 22 to 26°C entry into torpor is completed in three to four hours. During this period oxygen consumption may decline smoothly (Fig. 1) or it may show irregular excursions, presumably associated with body movements incidental to changes in posture (Fig. 2). In two of the five instances, the decline in oxygen consumption was preceded by a brief but conspicuous increase in metabolism.

Under the conditions of measurement of oxygen consumption it was not possible to observe behavior during entry into torpor. However, aestivating animals were observed almost daily in the laboratory during late summer, fall and winter. While entering torpidity, they assumed the usual sleeping posture with feet and head tucked under the body.

Torpor. Aestivating animals were most readily distinguished from sleeping animals by their respiratory pattern. Torpid animals showed prolonged periods of apnea, while sleeping animals did not. Oxygen consumption during torpor was usually extremely uniform and fell between 0.1 and 0.2 cc/gm/hr. There were, however, occasional increases in rate to as much as 0.4 cc/gm/hr. Under the conditions used for the measurement of oxygen consumption the duration of torpidity was variable, extending from eight hours to several days. Aestivating ground squirrels observed in the laboratory occasionally shifted position or changed posture without arousing.

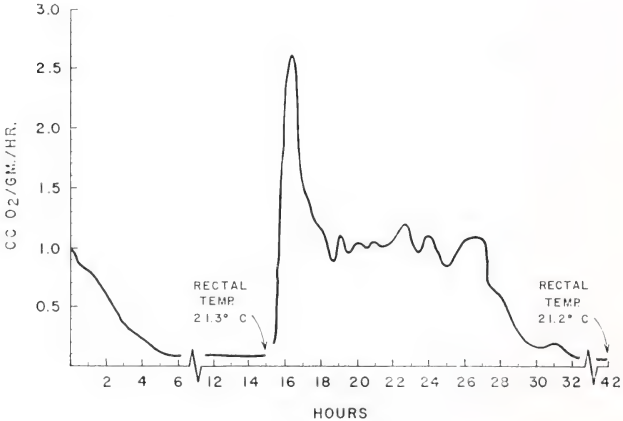

Fig. 1. Oxygen consumption during two entries into torpor, one induced arousal, and normal activity in an adult ♂ *C. mohavensis*, weighing 232 grams. Ambient temperature varied between 21 and 23.7°C.

During torpidity body temperature varied directly with air temperature and oral and rectal temperatures did not differ significantly from each other. Torpor was observed in animals at body temperatures ranging from 10.6 to 27.1°C. We do not know whether or not these represent the limits of body temperature at which animals of this species can remain torpid.

Arousal. When an aestivating Mohave ground squirrel starts to arouse, its oxygen consumption rises rapidly and may increase 10 to 20-fold in less than 15 minutes (Figs. 1 and 2). The peak of oxygen consumption is usually reached within 20 minutes of

the start of arousal. (As discussed below, body temperature increases much more slowly.) Oxygen consumption then declines to the normal resting level during a period of two hours or more. Thus, during much of the period of arousal from aestivation, oxygen consumption is actually decreasing from its initial peak while body temperature increases. As pointed out above, during aestivation the breathing of these animals is characterized by prolonged periods of apnea. With the onset of arousal, the breathing of an animal aestivating at 20°C or more immediately becomes continuous and within five to ten minutes reaches the normal rate of about 80 to 90 per minute; thereafter, it usually

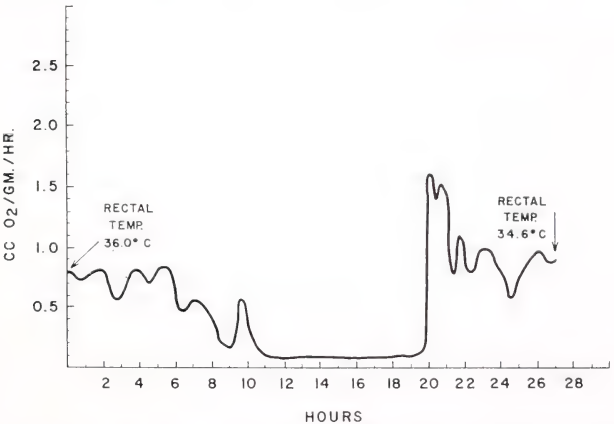

Fig. 2. Oxygen consumption during normal activity, entry into torpor, and spontaneous arousal in an adult ♀ *C. mohavensis*, weighing 306 grams. Ambient temperature varied between 23 and 26°C.

remains relatively constant in rate but may increase markedly in amplitude. At body temperatures below 20°C, periods of apnea continue to occur even while the animal is arousing. Toward the end of arousal the breathing rate sometimes becomes conspicuously depressed and the respiratory movements become very deep and heavy.

The time required for body temperature to rise to levels characteristic for normal activity depended largely on initial body temperature, but the rate of increase showed no significant cor-

relation with body temperature at start of arousal. Rate of temperature increase of the same animal during arousal varied from time to time (Fig. 3). At ambient temperatures between 22 and 27°C the maximum rate of increase in body temperature above ambient temperature was 0.4°C/min while the minimum rate was 0.1°C/min. The body temperatures at the termination of arousal showed considerable variation, extending between 33 and 38°C. In general, rectal temperature was the same as oral temperature at the beginning and end of arousal and

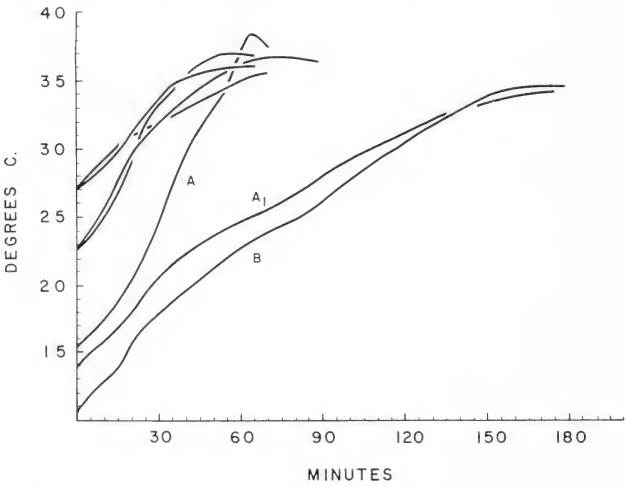

Fig. 3. Increases in body temperature during arousal in *C. mohavensis*. Lines A and A_1 represent different arousals by the same animal. Each of the other lines is for a different animal. Line B shows an arousal in which shivering was barely perceptible.

rarely was more than 0.5°C lower than oral temperature during arousal.

Characteristically, these ground squirrels shivered strongly during arousal. Occasional slight quivering of the anterior parts of the body were observed at body temperatures as low as 16°C. Strong, sustained shivering characteristically did not begin until body temperatures of 23 to 24°C were attained. Shivering appeared first anteriorly and then spread to the posterior parts of

the body. Strong shivering did not invariably occur during arousal although some shivering was always observed. Those animals showing least shivering had the slowest rates of increase of body temperature (Fig. 3). In no case did shivering continue after body temperature had reached 35°C.

Mohave ground squirrels with body temperatures as low as 10°C respond to touch by withdrawal. (Lower temperatures were not tested.) No vocalization could be elicited at body temperatures below 21°C, but it occurred in response to all disturbances at body temperatures above 25°C. At body temperatures below 15°C the animals were unable to right themselves when placed on their backs. With a body temperature of 20°C, the animals were capable of poorly coordinated crawling and slow, jerky walking. At this temperature they often attempted to dig. At 27 to 28°C their walking and digging activities appeared normal and coordinated. By the time body temperature reached 32 to 33°C behavior appeared normal in all respects.

Discussion

Aestivation versus hibernation. Long usage has established the word "aestivation" for summer dormancy in mammals as well as in other organisms. Although there can be no doubt that aestivation occurs naturally in mammals, particularly in regions of seasonal drought, the evidence for it is largely circumstantial — many species of rodents are not active above ground during parts of the dry season, and it is assumed that they are dormant. (See Kalabukhov, 1956, for a detailed review.) However, detailed physiological data on aestivation in placental mammals are limited to two genera, *Citellus* and *Perognathus*. Studies on *Perognathus longimembris* (Bartholomew and Cade, 1957) led to the conclusion that in this species aestivation and hibernation are the same physiological phenomenon, the only difference between the two being the level of body temperature, and this is dependent on ambient temperature. A similar conclusion seems justified in the one bird for which data are available: the Poor-will, *Phalaenoptilus nuttallii*, will become torpid over a range of environmental temperatures extending at least from 2° to 19°C (Howell and Bartholomew, 1959).

In the present study we have found that in the Mohave ground squirrel aestivation shows the classical criteria of hibernation: (1) body temperature within a degree or less of ambient temperature; (2) oxygen consumption markedly reduced; (3) prolonged

periods of apnea; (4) a torpor more pronounced than deep sleep; (5) arousal, either spontaneous or induced, accompanied by activation of the major heat producing mechanisms. Furthermore, the physiological and behavioral performance of this animal is qualitatively similar over body temperatures ranging from 10°C to 27°C. The physiological and behavioral differences between aestivating and hibernating Mohave ground squirrels are matters of degree and appear to be simply functions of body temperature, the level of which is determined by environmental temperature.

We, therefore, suggest that the terms aestivation and hibernation should be used only in the context of natural history to describe the summer and winter dormancy of warm-blooded animals. We feel that some other word or phrase, such as "facultative hypothermia," should be used for the physiological aspects of these phenomena in birds and mammals. Such a change in terminology would have the advantage of distinguishing between the performance of warm-blooded animals and all other organisms, would emphasize the close physiological similarity of summer and winter dormancy in a variety of mammals and birds, and could in addition encompass the "partial hibernation" of bears and the daily torpor of bats and hummingbirds.

Physiology. The capacity of various species of *Citellus* to become dormant at temperatures between 20 and 30°C offers the opportunity for further insights into the mechanisms of seasonal hypothermia in mammals. In mammals dormant at low temperatures (i.e. in the "deep hibernation" of Lyman, 1948, p. 56) the peak of oxygen consumption is not reached for more than an hour after the onset of arousal. However, in Mohave ground squirrels dormant at temperatures above 20°C, oxygen consumption may reach its maximum within 15 or 20 minutes from the onset of arousal. We interpret this to indicate that initiation of arousal is under the control of the central nervous system and that the rate of heat production is limited by cell temperature and, of course, modified by the condition of the animal. In an animal dormant at relatively high temperatures, oxygen consumption during arousal can increase more rapidly than in an animal dormant at low temperature because the metabolically depressing effects of low cell temperature are less. The behavioral responses of Mohave ground squirrels at a body temperature of 20°C are sufficiently complex and coordinated that one can infer a complex level of cortical activity at this temperature even in the absence of direct measurement. Our data did not show peaks of oxygen consumption during arousal as high as those reported

for some other species of *Citellus* (see, for example, Popovic, 1957); the general pattern, however, of an overshoot in oxygen consumption followed by a slow decline was the same in our animals as in ground squirrels arousing from body temperatures near 0°C.

In most hibernating mammals during arousal the increase in rectal temperature lags far behind the increase in oral temperature. This is usually not the case in the arousal of aestivating Mohave ground squirrels. They have, however, the necessary cardiovascular mechanisms for the establishment of this temperature differential. During an arousal from 16°C one individual had a rectal temperature of only 25°C when its oral temperature reached 35°C. The failure to maintain a marked antero-posterior temperature difference may be associated with the relatively high body temperatures at the onset of arousal in the present experiments.

In captivity with food continuously available, Mohave ground squirrels show intermittent periods of dormancy in all seasons except spring and early summer. We assume, therefore, that for months at a time they are in condition to aestivate or hibernate and that during this period no intervals of transition or physiological adjustment between normal body temperature and marked hypothermia are necessary. Entry into torpor is rapid and essentially unbroken, although the process may sometimes be interrupted. The rapidity with which this species can reduce its rate of oxygen consumption and allow its body temperature to drop to ambient temperature leads us to assume that initiation of the reduction of metabolism like the initiation of arousal is under central nervous control. Once metabolic rate has been reduced, body temperature declines with the end point being determined by ambient temperature.

Since the behavior of these animals is essentially normal at a body temperature of 32°C and since they can become dormant at a body temperature at least as high as 27°C, the difference between the minimum temperature of normal activity and the maximum temperature of aestivation is no greater than the range of body temperatures observed in normally active animals. This difference may in fact be even less than 5°, for Popovic and Popovic (1956) report that *C. citellus* can become dormant at 30°C. Viewed thus, the transition from normal activity to dormancy represents only a minor change in body temperature, but represents a profound change in metabolic state. As previously discussed, the transition from aestivation to hibernation

is unbroken. Therefore, for months at a time this species is a facultative poikilotherm (or facultative homeotherm) and can have the metabolic advantages of both poikilothermy and homeothermy, as needed, to meet the demanding circumstances of its desert environment.

Ecology and Sympatry. Citellus mohavensis occurs only in the Mohave Desert of California, a region characterized by extremely hot, rainless summers and mild winters with light undependable precipitation. Although in winter nighttime temperatures may fall below freezing, the days are almost invariably mild and a diurnal rodent need never be exposed to prolonged low temperatures. Seasonal dormancy in the Mohave ground squirrel does not appear to be an adaptation for the avoidance of low environmental temperatures, but appears to be an adaptation to seasonally restricted food and water.

The range of *C. mohavensis* lies completely within the distribution of the Antelope ground squirrel, *Citellus* (*Ammospermophilus*) *leucurus*. The two species occur together in an extremely simple desert plant association. Their maintenance of sympatry appears to be most readily explicable in terms of the marked differences in their patterns of metabolism. *C. leucurus* neither aestivates nor hibernates but remains active above ground at all times of the year; we have been unable to induce dormancy in the laboratory. *C. mohavensis* stays underground and presumably dormant except during the most favorable part of the year — spring and early summer. Thus, in the area of sympatry during the more demanding and difficult parts of the year — late summer, fall, and early winter — only *C. leucurus* is active. Consequently, the two species compete for water (in the form of insects and succulent vegetation) and food only when supplies are maximal and presumably adequate for both. Thus, from the point of view of energetics, during the more difficult parts of the year only one species is present. It seems, therefore, reasonable to postulate that between these two sympatric ground squirrels competition, in the sense of utilization of a common resource which is in short supply (Birch, 1957, p. 6), is minimal and perhaps does not exist, except in very poor years, because of the differences in the seasonal patterns of their metabolism.

Patterns of Seasonal Dormancy in Citellus. The genus *Citellus* has a circumpolar distribution. Ellerman and Morrison-Scott (1951) recognize seven species in the Palaearctic; more than a score occur in the Nearctic and several occur in the Neotropics (Miller and Kellogg, 1955). Howell (1938) recognizes eight

subgenera. To date no demonstration of seasonal dormancy is available in the five species of the western North American subgenus *Ammospermophilus*, but hibernation, and less commonly aestivation, occur in all of the other subgenera with the possible exception of the neotropical subgenus *Notocitellus*. If one excludes *Ammospermophilus*, a general pattern for the occurrence of seasonal dormancy in ground squirrels suggests itself: (1) they hibernate where the winters are cold; (2) they aestivate in regions of prolonged seasonal drought; (3) aestivation merges into hibernation in northern arid regions where precipitation is seasonal and restricted to winter and spring; and (4) aestivation does not occur in areas of regular summer rainfall. Viewed in this perspective the seasonal dormancy of ground squirrels offers a primary physiological key to their abundance and success in a variety of habitats ranging from the tundra to subtropical deserts and covering over 50 degrees of latitude. The fact that this dormancy can occur from near 0° to 30°C and, therefore, is not closely dependent on environmental temperature allows various species of this genus to avoid those periods of the year during which drought, high temperature, low temperature, or availability of food is locally limiting. Thus, a single physiological capacity adapts this genus to the Arctic, to high mountains, and subtropical deserts.

The capacity for seasonal dormancy is of no *a priori* advantage in tropical regions in which seasonal drought is not severe. It is of interest that this genus has had very limited success in occupying the humid tropics.

Summary

The Mohave ground squirrel, which is confined to the Mohave Desert of California, is normally active above ground only during spring and early summer. Under laboratory conditions it spontaneously enters torpor at room temperature and spends much of the summer, fall, and winter in a dormant condition. During periods of normal activity its standard metabolism averages slightly more than 0.8 cc O_2/gm/hr and its body temperature averages slightly less than 36°C. The animals may become torpid over a range of ambient temperatures extending at least from 10 to 27°C. When entering torpor at ambient temperatures between 22 and 26°C they assume the usual sleeping posture, and their oxygen consumption declines rapidly and body temperature approximates environmental temperature within three or four hours. Thereafter, both oral and rectal temperatures vary

directly with ambient temperature. During torpor oxygen consumption is less than 0.2 cc/gm/hr.

Following the onset of arousal oxygen consumption increases 10 to 20-fold and usually peaks within 20 minutes. Body temperature increases more slowly, and the levels of body temperature characteristic of normal activity are usually attained in 45 to 60 minutes. Typically, rectal and oral temperatures are within 0.5°C of each other during arousal. The behavioral capacities of the animal increase steadily as body temperature rises and appear normal at 30°C.

Since aestivation and hibernation are physiologically similar in those few mammals and birds in which they have been compared, it is suggested that the two words be used only in a natural history context and that another term, perhaps facultative hypothermia, be used when dealing with the physiological aspects of the phenomenon.

The ecological roles of seasonal dormancy in the genus *Citellus* are surveyed and the role of dormancy in distribution and sympatry is discussed.

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DISCUSSION FOLLOWING BARTHOLOMEW'S PAPER

SOUTH "heartily endorsed" BARTHOLOMEW'S point of view except for one matter: he noted that in dealing with clinicians there already is confusion in the use of the word "hibernation," that he would anticipate still further difficulties when scientists tried to make their interests understood by clinicians if the phrase "facultative hypothermia" were used. BARTHOLOMEW noted that, since temperature is the most obvious parameter measured, the phrase "facultative hypothermia" would be logical.

SOUTH agreed that BARTHOLOMEW'S approach made sense. BARTHOLOMEW added further that the use of this phrase would allow scientists to get away from many problems; for instance, it would permit seasonal falls in body temperature change to be related to "hypothermia" and not to "hibernation;" it would give a convenient phrase for referring to conditions such as partial or "shallow" hibernation and daily torpidity.

FOLK asked BARTHOLOMEW to discuss the oxygen consumption of a lizard, frog, and mammal of the same size at 22°C in terms of the "thermostat" concept. BARTHOLOMEW noted that the word "thermostat" should be used as a symbol for a complex physiological mechanism. "Facultative hypothermic" mammals have oxygen consumptions at body temperatures of 22°C which are not greatly different from those of medium-sized lizards or big frogs at that temperature. What seems to be essential, BARTHOLOMEW observed, is as MORRISON said — that such a mammal has reset its "thermostat." This, of course, does not explain the process; it is possible that muscle activity is completely, or almost completely, cut off and that this relaxed muscle is simply not producing heat. This, he

said, was as far as he could go with this explanation. The "thermostat" concept does not demand an elaborate reorientation of body chemistry or reinterpretation of what goes on in the central nervous system.

PEARSON asked if it was not true that an "obscure physiologist named LYMAN" had shown that heart rate decreased first, and that then the body temperature went down as an animal went into hibernation. He noted that this does not fit into the "thermostat" concept. LYMAN disagreed by pointing out that the heart could be "turned off" first. BARTHOLOMEW noted that as long as the phenomenon is characterized by a word like "thermostat" one does not have to say what the "thermostat" is.

SMITH then pointed out that "setting" the thermostat at a lower level was a valid proposition, but when one considered the resting bat, the body temperature of which follows the environmental temperature, difficulty arose. BARTHOLOMEW said that he did not feel the difficulty was great, but there was "drift" in the system if the animal were not regulating its temperature closely. MORRISON remarked that this was not too easy for non-hibernators. BARTHOLOMEW generalized by saying that the "thermostat" concept allows one to relate many phenomena to the same mechanism.

HOCK then indicated that he believed the "thermostat" concept was a simplification of a mechanism about which we were fairly ignorant, and it may very well be equally feasible to consider that the animal is not "turning off" anything whatever, but rather "turning something on." Turning off a thermostat or "drifting down" may be incorrect. Observed phenomena might not represent passive abandonment, but rather an active additional mechanism. This, of course, may vary with species. Animals which move from one physiological state to another generally do so in a positive way. BARTHOLOMEW remarked that this may apply to animals that go to low temperatures. The Mohave ground squirrel, on the other hand, showed virtually normal movements at 32°, but could become dormant at 27°C. He said he felt there is a passive drift once this condition (dormancy) is reached. What happens below 10°C, he could not say, but each genus and possibly each species has its own particular pattern.

PROSSER stated that all of these concepts of a thermostat are as broad and perhaps more vague than "centrencephalic control of autonomic function." He indicated he would not suppose all of this could occur in one small bit of the hypothalamus, but rather in whole portions of the brain. BARTHOLOMEW said that if one could find a "trigger" which set off the whole mechanism, everyone would be happy.

WIMSATT asked if there were any cases in which body temperature dropped below ambient temperature. BARTHOLOMEW said the only way this could be done would be through evaporative water loss, and this would be metabolically expensive.

MORRISON stated that one cannot speak of a positive regulation in the thirteen-lined ground squirrel in which the body temperature follows the varying ambient temperature within a degree. Obviously some animals such as the hedgehog do regulate when the temperature becomes too low.

HOCK commented that this animal is not passively turning off anything, but just resetting his thermostat. BARTHOLOMEW said that he agreed with MORRISON; if an animal is not regulating, it drifts to the ambient temperature. This idea of allowing body temperature to drop to the environmental temperature encompasses bats, hummingbirds, etc.

LANDAU asked why the heart does not slow before the breathing rate. BARTHOLOMEW replied that this question will be answered when one knows what is triggered, and what is doing the triggering. MORRISON pointed out that where heart rate slows first, it can be due to the fact that hibernation may first be preceded by sleep, at which time the heart slows, and that then the animal goes into the hibernation stage. It is usually a requirement that sleep precede hibernation.

POPOVIC then noted he had done experiments which would be in line with BARTHOLOMEW'S observations on correlations between hibernation and aestivation. He (POPOVIC) said they kept ground squirrels in a Herter apparatus, and the animals hibernated when they could choose the temperature of the room. The animal chose a 28°-30°C room to hibernate during the winter. Also, the color-marked male and female ground squirrels in the field showed that the males disappeared first, entering hibernation at the end of August, which is the hottest month in Yugoslavia.

DAWE returned to the question of the "thermostat." He noted that if the hibernating ground squirrel is decapitated, and the heart removed and placed in cold saline, it continues to beat. If the heart is removed from an active animal (which normally hibernates) in a non-hibernating "season," it usually stops quickly in cold saline. This difference in the tissue is not explainable on the basis of a simple reset of a thermostat. BARTHOLOMEW replied that he thought the entire animal changed truly enough, but the critical thing in the present discussion, it seems, is to consider how he does get from one state to another, and what adjustments he is making.

MORRISON concluded the discussion by stating that he agreed entirely with BARTHOLOMEW'S concept of the identity of the dormant states of hibernation and aestivation, but he felt the term "facultative hypothermia," although quite proper, would be difficult to substitute for the older terms. He suggested as an alternative that the terms "hibernation" and "aestivation" continue to be used, but with general recognition of their physiological identity. In most cases, he said, the respective word could describe the animal or circumstances and there would be a negligible number of cases in which the choice would be difficult.

XI

DAY-NIGHT RHYTHMS AND HIBERNATION[1]

By G. Edgar Folk, Jr.
Department of Physiology
State University of Iowa
Iowa City, Iowa

Introduction

Biologists have recognized for many years the presence of 24-hour rhythms of physiological activity or movements in plants and animals. These rhythms appear to have originated because of the day-night light cycle. Many animals and plants utilize the light cycle by accepting dawn or sunset as a clue to keep their rhythms synchronized with solar time (Pittendrigh and Bruce, 1957). When many plants and animals are placed in continuous darkness, the biological rhythms persist, sometimes on a 24-hour basis but also at times with a "period" which is a few minutes (up to an hour) more or less than 24 hours. Many investigators believe that a physiological interval-timer, or innate physiological periodicity of about 24 hours, is as much a fundamental characteristic of animals as certain oft-repeated organ patterns such as nervous systems and brains, or circulatory systems and hearts. A characteristic of these rhythms is their temperature independence. Such a phenomenon demands the attention of biologists, because any presumed biological clock which controls periodic recurrence of physiological functions with the accurate timing observable in biological rhythms, must depend for its mechanism upon the activity of enzymes. One would predict that just as the activity of brain, heart and muscle tissue is reduced at cold temperatures, likewise the biological clock would run more slowly. The problem encompassed by the present report is a search for marked persistent 24-hour rhythms of mammals when their body temperatures are reduced to about 5°C. Since there is admittedly little information on mammals, let us first consider temperature independent rhythms as found in plants, one-celled organisms, fiddler crabs, and lizards. An illustration of a rhythm of growth in plant tissue is found in the work of Ball *et al.* (1956) (Fig. 1). When seedlings are trans-

[1] This research was supported by the National Science Foundation.

ferred from red light to darkness they do not continue to grow at a constant rate but show two or three 24-hour rhythms of high and low growth rates. The amplitude of this rhythm changes at a cold temperature, but the timing of the peak of activity remains essentially unchanged. The second example is seen in *Euglena*, as studied by Bruce and Pittendrigh (1956)

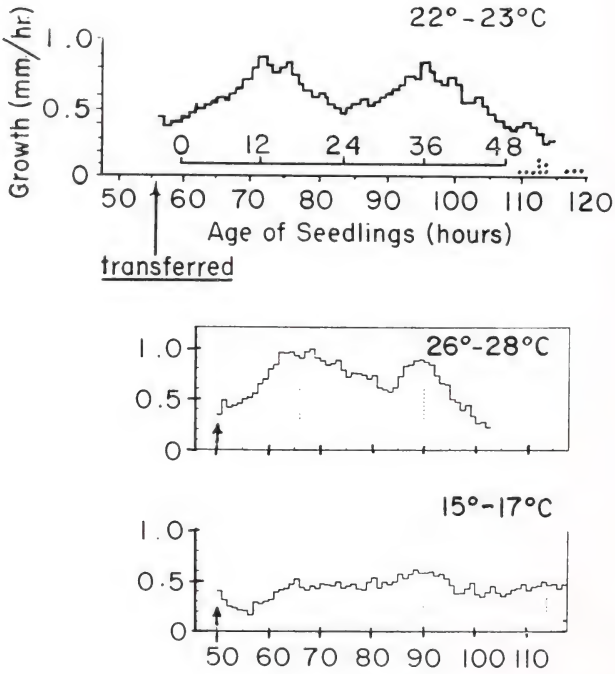

Fig. 1. Mean growth rate of *Avena* coleoptiles in darkness. Seedlings transferred from red light to darkness at the 50th or 56th hour from soaking. Times of emergence of primary leaves are indicated by dots. Three experiments demonstrating endogenous rhythms are shown. At the lower temperature (15°-17°C) the amplitude of the rhythm is low, but the period of the rhythm is the same. Diagrams reproduced from work summarized in Ball, Dyke and Wilkins (1956).

(Fig. 2). These organisms orient to light for about 12 hours and fail to respond for about 12 hours, showing a 24-hour rhythm in this respect. One might expect that when the environmental temperature is changed from 33°C to 23°C the 24-hour rhythm might become one of about 28 or 30 hours. However, there is no change at 23°C and very little change at 17°C. Turning now to the Crustacea, we find that a central hormonal mechanism darkens the color of fiddler crabs for a period of about 12 hours, so that the shell of these animals will more nearly match their

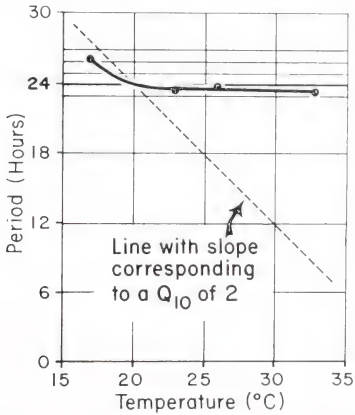

Fig. 2. Temperature-independence in persistent rhythm of phototaxis in *Euglena gracilis*. From Bruce and Pittendrigh (1956).

surroundings in the daytime and will provide protection against the sun's rays. Brown and Webb (1948) showed that this central mechanism is independent of temperature (Fig. 3). As usual the amplitude of the response is lowered but the timing remains approximately unchanged. Kayser (1952) has observed the same phenomenon in the locomotor activity of the lizard, when either oxygen consumption or total activity is recorded (Fig. 4). The *quantity* of activity follows the law of Arrhenius, and shows a reduction at low temperature, but the *time* of the activity in continuous darkness is unchanged by low temperature. When the lizard must "meet a friend at the bank" at 12 o'clock on a hot day he trots vigorously and briskly to arrive on time at the destination. If the day is cold he sluggishly and laboriously lifts

one foot at a time, but arrives at the destination as usual at the appointed hour.

When the mammal is considered, a special problem arises. Hibernators and other mammals in hypothermia (including man) experience a reduced metabolism and body temperature.

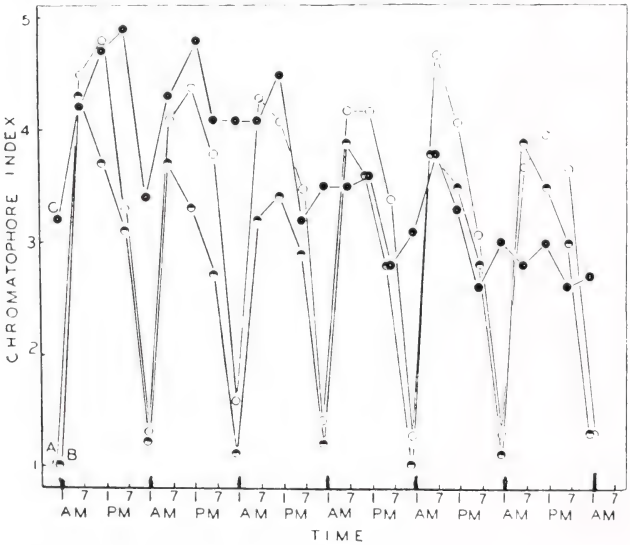

Fig. 3. Daily variation in the average indices of black chromatophores in the fiddler crab, *Uca*, kept in constant darkness at 26°C (A), 16°C (B), and 6°C (C). The temperature independence of the rhythm of color change is apparent. From Brown and Webb (1948).

When body temperatures are normal some of the most accurate and regular of biological rhythms are found in mammals. Compare the running wheel of thirteen-lined ground squirrels (day-active), and rats and hamsters in Figure 5 remembering that two of these rodents are hibernators. Mammals and birds must require a narrow range of variation in their blood temperature, evidenced by the fine and sensitive control found in their physiological thermostat (the hypothalamus). Perhaps as a result of

this requirement, the biological clock of the mammal cannot function when it is cold. On the other hand, there may be the same mechanism for temperature compensation in the mammal that has just been illustrated in plants, one-celled organisms and other ectothermic animals. Some experimental data will be presented in support of the second of these two alternatives.

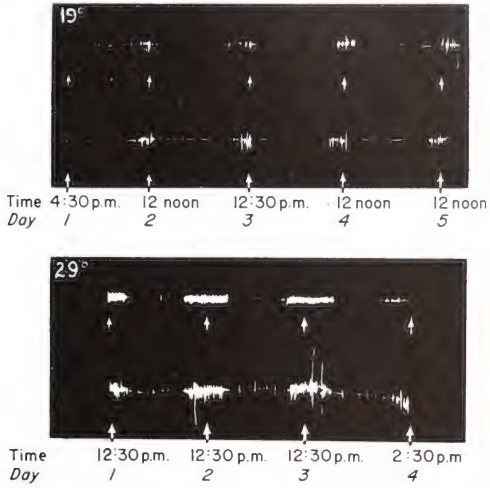

Fig. 4. Day-night rhythm of activity of the lizard. From Kayser (1952).

Methods

Studies on ground squirrels. A colony of ground squirrels (*Citellus tridecemlineatus*) was maintained for three years with intermittent periods of 8 months at a warm temperature and 4 months of hibernation in the cold. The detailed treatment of the 11 experimental ground squirrels and the 14 control ground squirrels (no hibernation) has been presented earlier (Folk, 1957). During the periods of hibernation each winter, observations were made twice a day. The animals were marked with oats or sawdust which fell off or was cleaned off when the animals awoke from hibernation. Actographs were not considered appropriate here because animals have been known to remain awake but unmoving for reasonably long periods even

in darkness. In another series of experiments, body temperatures were taken by hand at selected times of the day using a thermistor and a telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio). Skin temperatures were measured with calibrated copper-constantan thermocouples. Heart rates were measured with a Burdick electrocardiograph. For 24-hour measurements the instrument was turned on automatically every half hour for two minutes.

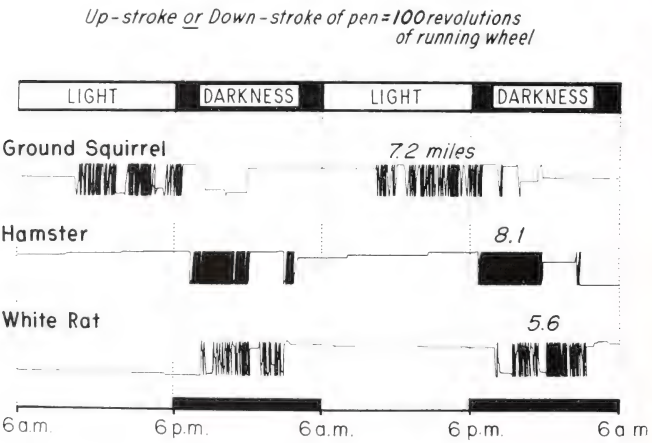

Fig. 5. 48-hour records of spontaneous running of three species of rodents. The times of starting of activity are very regular. The ground squirrel is day-active but continues to run after the dark period begins. The hamster is nocturnal but usually is through running by 3 a.m. Activity was measured with Welsh recorders.

The 1957 series of squirrels was sacrificed to obtain physiological and anatomical data (11 hibernators, 14 controls). The 1958 series (heart-rate series) consisted of five ground squirrels studied in a two-door refrigerator rather than in a cold room. The test chamber was in a water bath in the refrigerator, which was illuminated from 9 a.m. to 9 p.m. (2 foot candles). During four months of cold exposure, one animal did not hibernate; of the others, three were light hibernators with short periods of hibernation, and the remaining animal was a deep hibernator

with extremely long bouts of hibernation lasting weeks at a time (Fig. 6).

Studies on bats. A series of studies was done on 16 hibernating bats (*Eptesicus fuscus*), since they have been shown to have at normal temperatures a 24-hour rhythm of activity, both with a light cycle and in continuous darkness. Actographs are not always satisfactory, and so a stimulus test and index, described

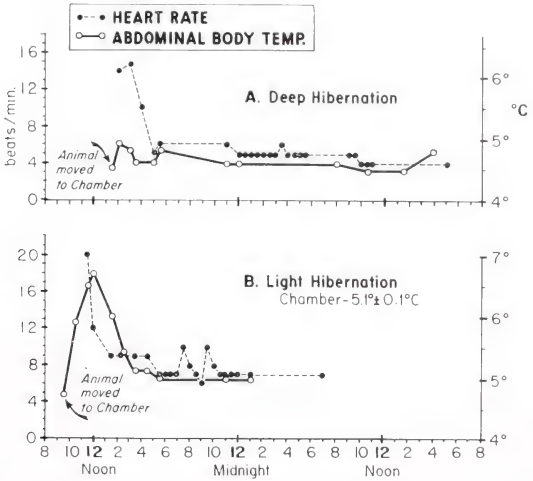

Fig. 6. Heart rate and temperature of ground squirrels in light and deep hibernation. The deep-hibernator (A) usually had a heart rate of 4/minute (4.5°C), and the light-hibernator (B) had a rate of 7/minute (5.0°C). Hibernation before these readings for animal *A* was 13 days, after these readings, 8 days; hibernation before readings for animal *B* was 3 days, after readings, 2 days.

earlier, were used again (Folk, 1957). The method involved a stimulus consisting of a standardized, mechanical puff of air at 6 inches. This was applied daily or hourly to test the hibernating condition of each bat. Series A consisted of giving the stimulus every 25 hours so that a 24-hour rhythm would not be established; Series B consisted of testing each member of the colony every 2 hours over a 24-hour period (both series new, 1959). The animals, each in a separate cage, were given the stimuli quickly

when the refrigerator door was opened briefly. Toenails and hibernating position were marked with yellow paint. Each animal also received the stimulus of the weak light of a flashlight used for observational purposes. The animals were maintained in continuous darkness in hibernation for three winter months.

Results

Regularity in periodic awakening of ground squirrels from hibernation. Most of the awakenings during hibernation, in the second and third winter, of 11 hibernating ground squirrels occurred during the simulated daylight. This pattern of awakening was more marked during the second winter (72 per cent of the time during the periods corresponding to the original light cycle). The awakening pattern was also evident during the third winter of hibernation, in observations on the same squirrels. The animals were illuminated in the cold room daily from 9 a.m. to 9 p.m. and only at these times were observations made.

TABLE I.
Effect of Three Winters of Hibernation
on 13-Lined Ground Squirrels.

	Males		Females		Mean ♂ and ♀	
	CONTROL	HIBERN.	CONTROL	HIBERN.	CONTROL	HIBERN.
Weight (gms.)	161.9	147.0	167.4	100.3	164.7	123.7
Body Temperature (°C)	36.1°	36.1°	36.4°	37.1°	36.3°	36.6°
O_2 Consumption (ml/gm/hr.)	0.769	1.027	0.980	1.535	0.874	1.281
Spleen (gms.)	.180	.151	.193	.127	.187	.139
Heart (gms.)	.837	.980	.715	.771	.776	.876
Kidney (gms.)	.495	.561	.380	.381	.438	.471
Testis (gms.)	0.261	1.136	--	--	0.261	1.136
Hematocrit (%)	--	--	--	--	59%	51%
Lipemia Index	--	--	--	--	2.0	2.6
Coagulation Time (%)	--	--	--	--	Con. 108% of Hib.	

Body temperatures and O_2 consumption were measured at 30°C. Note the loss in weight of spleens and gain of hearts and kidneys. Other studies have shown that this effect on the last two organs is a true hypertrophy.

At the end of the winter the animals were taken from hibernation, permitted to warm for six hours and sacrificed. A series of measurements was compared with those of controls (Table I). All eleven animals had hibernated intermittently for the four months, awakening after intervals of dormancy lasting from one to 26 days; 164 periods of dormancy were observed (Table II). Again, for the second season, more squirrels (63 per cent) awoke from the dormant condition when the light was on (Table III). If the hypothesis is tested that light or some light associated phenomenon caused the awakenings in light, then they are

TABLE II

Hibernating Data for 13-Lined Ground Squirrels During the Third Winter. N = 11. Ambient: 6 ±1°C

	Total hibernating days	Total hibernating periods (bouts)	Range of period length in days	
			minimum	maximum
October	218	51	1.0	17.0
November	297	37	1.0	22.5
December	353	32	3.5	26.0*
January	294	35**	2.0	22.5

* This maximum hibernating period is the longest recorded in three years of study on these squirrels.
** There were 9 additional periods in February.

significantly different from the awakenings in darkness. One individual always awoke during the illuminated period, while a few awoke most of the time in darkness. Two interpretations of this regular awakening would be: (1) that the animals, in spite of a reduced metabolism and body temperature, still received some stimuli from the external environment which could act as clues to cause the periodic awakening; or (2) that some 24-hour rhythm was still expressed in the animals in such a way as to influence their awakening from hibernation. One might postulate light and deep hibernation corresponding to the regular periods of sleep and wakefulness which occurred before hibernation. If this were so then the usual or typical stimuli which cause awakening from hibernation such as hunger or a full bladder, might be more apt to take effect during the period of light hibernation. The experiments to determine whether the regular awakening was due to external stimuli will be described first. It appeared unlikely that any artificial light

could penetrate to the eyes of a hibernating ground squirrel since the head was invariably buried beneath the body with the eyelids closed. In addition, there is no evidence that the optic nerve can conduct when maintained at a temperature near 5°C. It was considered most profitable to first design experiments on hibernating ground squirrels with "white noise" as a stimulus.[2] Four series of tests were tried with three hibernating

TABLE III

Distribution of Awakenings of Dormant 13-Lined Ground Squirrels During a 4-Month Hibernating Period.

Animal	DARKNESS 12 hours	LIGHT 12 hours
1	5	8
2	11	8
3	4	7
4	3	9
5	5	10
6	11	5
7	6	14
8	4	10
9	-	10
10	3	11
11	9	4
	61	96
	157	

*Statistical Analysis**

$\Sigma D = 35$

$\overline{D} = +3.18$

$\Sigma D^2 = 413$

$(\Sigma D)^2/n = 111.364$

$\Sigma d^2 = 301.636$

$s_D^2 = 27.421$

$s_D = 5.24$

$\sigma_{\overline{D}} = 1.66$

$t = \frac{3.18}{1.66} = 1.92$

$t_{5\%}\,(df=10) = 1.81$

(One-ended Test)

* Courtesy Prof. P. Blommers, State University of Iowa.

Note that one animal always awoke during the illuminated period. Long and short bouts of hibernation were found with most animals, and length of dormancy was unrelated to time of awakening.

[2] These experiments were made possible by the generous assistance of two staff members in the Department of Otolaryngology: Dr. J. Tonndorf designed the experiment and loaned the equipment; Dr. R. Voots engineered the electrical circuits.

ground squirrels (Table IV). The experiment was planned to produce repeatedly a loud noise such as to cause a "startle response" in non-hibernating ground squirrels. In each series all animals had hibernated for one day or longer, before the noise was applied in the refrigerator. In the first experiment, two animals were not hibernating. They were observed through a

TABLE IV

Experiments Applying White Noise as a Stimulus to Hibernating Ground Squirrels. N = 3

Experiment	Stimulus characteristics	Duration	Length of hibernation after "white noise" Animal #
1	90 db. 2 sec. on 2 sec. off	140 mins. (2.3 hrs.)	1 . . . . . over 24 hrs. 2 . . . . . over 24 hrs.
2	90 db. 2 sec. on 2 sec. off	60 mins. (1 hr.)	1 . . . . . over 24 hrs. 2 . . . . . over 24 hrs. 3 . . . . . over 24 hrs.
3	90 db. 2 sec. on 2 sec. off	240 mins. (4 hrs.)	1 . . . . . over 24 hrs. 2 . . . . . over 24 hrs. 3 . . . . . Awoke 2 hrs. after noise stopped. (This response within control awakening times, 1-3 days.)
4	97 db. 5.5 sec. on 9.0 sec. off	320 mins. (5.3 hrs.)	1 . . . . . over 24 hrs. 2 . . . . . over 24 hrs. 3 . . . . . Awoke 20 minutes after noise started. Had hibernated unusually long (4 days).

thermopane in the door. The expected startle response was easily observed, demonstrated by a characteristic head jerking when the noise first came on which was not continued throughout the application of the noise. The other two animals did not awaken from hibernation. In the second and third experiments, the hibernators appeared to be uninfluenced by the sound. During the third experiment heart rates were measured on one animal

(Fig. 7). The heart rate did not change from control values. In the last experiment the intensity of the sound was increased from 90 db to 97 db. Although one animal awoke 30 minutes after the noise began (respirations 18/minute), it is impossible to know whether this awakening was spontaneous or not. This animal had been hibernating an unusually long time. The other two animals were undisturbed by the noise. The conclusion is that these ground squirrels are seldom, if ever, awakened from

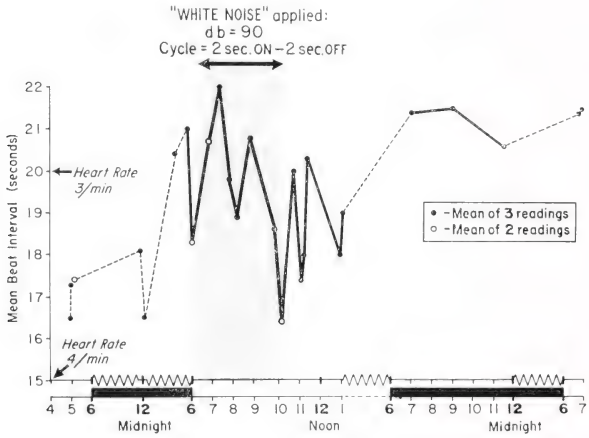

Fig. 7. Study of hibernating ground squirrel exposed to light and "white noise." The heart rate during the application of noise stayed within control values. Later, over 24 hours, the beat gradually became slower. There was no evidence of any effect of noise upon the hibernating animal.

hibernation by noise. It could hardly be a factor in influencing the observed regular awakening from hibernation.

Experiments on variation in depth of hibernation. Heart rates on the ground squirrel which showed deep hibernation were repeatedly measured over 24-hour periods (1958 series). There was slight evidence of any change in heart rate during day and night (Fig. 8). Few measurements could be obtained on the light-hibernators since they usually awakened when electrodes were attached to the chronically implanted surgical clips. When measurements were made on these animals, hibernating heart

rates were higher than those of the single animal in deep hibernation. One series indicated a heart rate level around seven per minute with a skin temperature of 5°C for the light-hibernator, and a heart rate level of 4 per minute and a skin temperature of 4.5°C for the deep hibernator (Fig. 6). Since it was possible that there might be a difference in the heart rate interval in day and night, this was studied on one animal in deep hibernation. The evidence indicates that there are frequent occurrences of

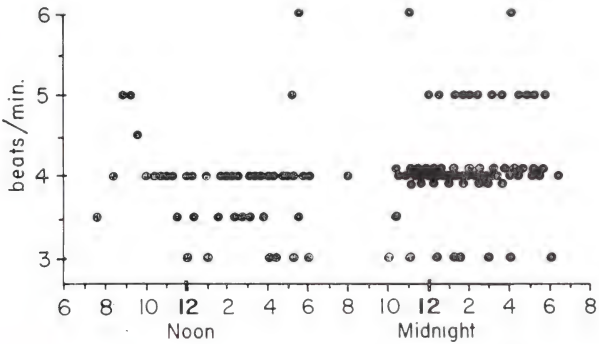

Fig. 8. Heart rates of hibernating ground squirrel sampled each half hour for three consecutive days. There was no evidence of a persistent 24-hour rhythm of heart rate, nor of any similar rhythm of approximately 23 or 25 hours. The entire 24-hour day was scanned, but in some cases instrument failure prevented 3 repeat readings.

regular and irregular heart beats distributed equally over day and night (Fig. 9). There was little evidence from the overall series of experiments for regular variations in depth of hibernation. Only in one experiment was there any clue at all; in this case body temperature readings showed spontaneous partial rewarming. Temperatures were taken day and night for six days, although no heart-rate measurements could be made at this time (Fig. 10). On the 7th day there was a partial temporary rewarming, following which the animal returned to deep hibernation. The fact that spontaneous rewarming may have begun in the light is not important in a single occurrence of this sort.

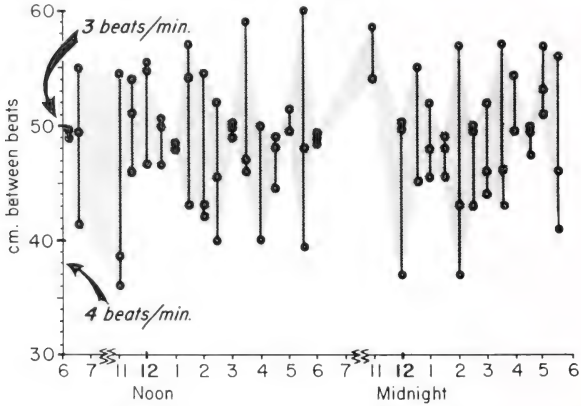

Fig. 9. Consecutive heart beat intervals of ground squirrel in hibernation. This analysis was made to reveal a 24-hour rhythm in heart beat regularity. There was no apparent difference between day and night readings. Likewise, the mean heart rate was unvarying over 24 hours.

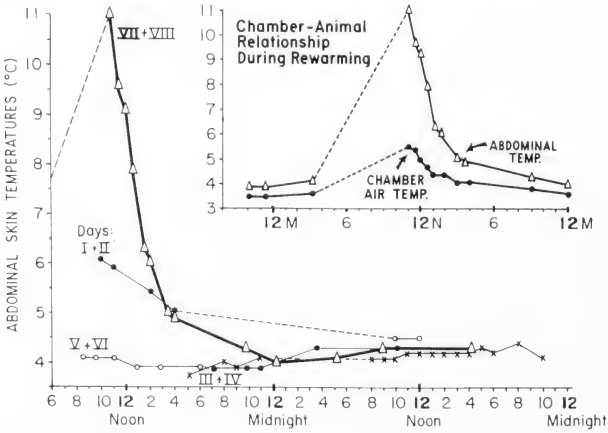

Fig. 10. Spontaneous partial rewarming (Day VII) from hibernation. This animal was studied for eight consecutive days, using skin temperature as an index to reveal day-night differences. For five days the temperature index showed a constant level of hibernation. There was one partial rewarming, during which the animal did not awaken from hibernation. Visual observations were made prior to temperature records on Day VII. No heart rates were taken.

Experiments on bats. Regular observations on 16 hibernating bats (1959 series) showed no particular time of the day when these animals arose from dormancy to drink or become active. As an earlier experiment showed (1957 series), the dominant effect was one of constancy. Some individual animals remained dormant for periods of days (Figs. 11, 12). Three did not even move their

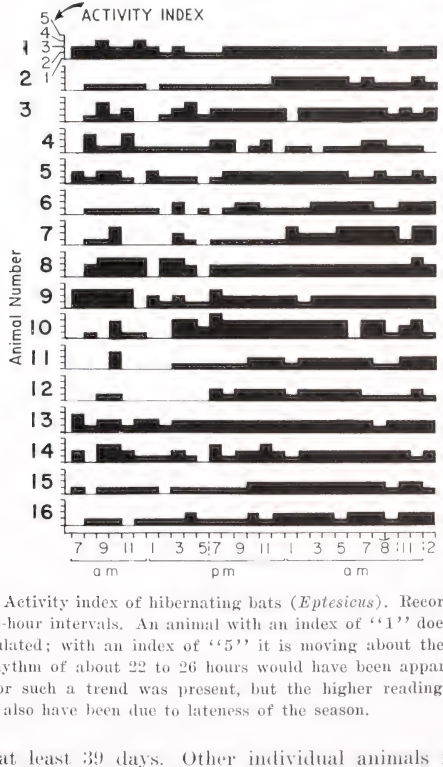

Fig. 11. Activity index of hibernating bats (*Eptesicus*). Recordings were made at 25-hour intervals. An animal with an index of "1" does not move when stimulated; with an index of "5" it is moving about the cage. An inherent rhythm of about 22 to 26 hours would have been apparent. Some evidence for such a trend was present, but the higher readings after 15 days could also have been due to lateness of the season.

feet for at least 39 days. Other individual animals remained semi-dormant for the total period of time and appeared to come down from their hibernating positions at irregular intervals.

Over any selected 24-hour period for each individual animal there was no obvious tendency for the animals to be in lighter hibernation for a portion of this period. The colony as a whole appeared to be in lighter hibernation when readings were made

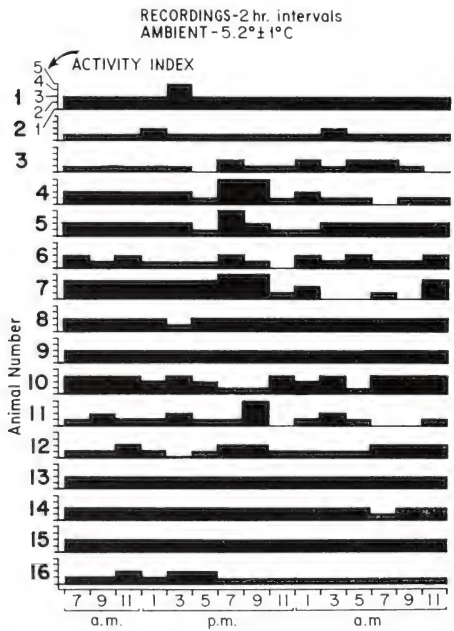

Fig. 12. Activity index of hibernating bats (*Eptesicus*). Recordings were made at 2-hour intervals, after the long series (25-hour intervals) was completed. As in the other experiment, there is some evidence for increased activity in the evening (see Table V).

at 4 a.m. (mean index 3.00) (5 p.m., index 1.85), as if a number of individual rhythms of activity had come into phase during the night period (Table V). If this were so, it would be a persistent rhythm, since there was no light cycle in this experiment. Although it can be said that there is little evidence of a clock mechanism in bat activity at a reduced body temperature, at a

warmer temperature this phenomenon must be important. Hock (1951) has shown that the bat is the only mammal in which body temperature is a direct function of environmental temperature in the resting animal. It will be noted from the actograms of Griffin and Welsh (1937) (Fig. 13) that for about half of the day, with either the light cycle or in darkness, the animal is inactive. We have shown in our laboratory that if the animal is maintained at 19°C its esophageal temperature during activity may be 29.1°C, but when at rest its temperature drops to 20.5°C.

TABLE V

Day-Night Differences in Mean Colony Dormancy Index of Hibernating Bats (*Eptesicus*). N = 16

Method of recording	Time	Mean index	p Value
Exp. 1 Every 25 hours for 29 days	5 p.m.	1.81	1%
	4 a.m.	3.00	
Exp. 2 Every 2 hours for 28 hours. (performed after Exp. 1)	5 p.m.	2.56	> 5%
	9 p.m.	3.06	

Thus, during its resting period any hypothetical biological clock was perfused by blood at a temperature 8.6 degrees lower than that found in the active bat.[3] Apparently from the regularity of the actograms of Griffin and Welsh, the bat behaves in the same way as Kayser's lizards (1952). The clock corrects for the drop of 9°C.

Other observations of mammalian physiological activity independent of temperature. The case for the mammalian biological clock at low body temperatures receives supporting evidence from other experiments. If we ask whether the performance of some mammalian nerves remains unchanged at different temperatures, we can cite the experiments of Kahana *et al.* (1950), who found that the neural component of the cochlea potential of the

[3] The drop in body temperature of the bat mentioned above must influence many experiments. In the experiments on bat rabies being conducted in the U. S., it is not commonly recognized that the rabies virus is not being maintained by the animal at a constant temperature of approximately 38°C. Instead, for half of the day the virus is "incubated" at whatever the animal-room temperature happens to be. Perhaps this phenomenon helps to explain why some individual bats are symptomless transmitters of the rabies virus.

hamster in the range 39°C to 25°C showed no change in its amplitude. More evidence for the temperature independent biological clock was also contributed by Rawson (personal communication) who showed that when activity records were made for bats and mice, and then the animals were kept under anesthesia and a reduced body temperature for several hours and returned to the activity recorder, their activity occurred at the appropriate time as if body functions had not been reduced or altered by the period of anesthesia. Finally, Strumwasser (1959) found that *Citellus beecheyi* "measured preferred times" for arousal from hibernation at body temperatures lower than 23°C.

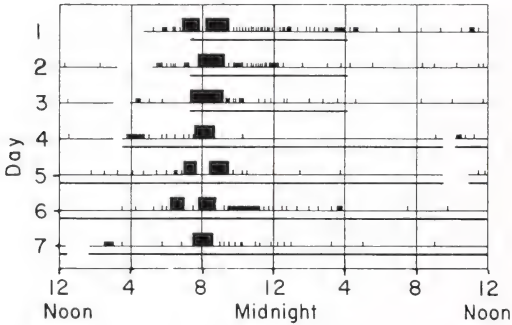

Fig. 13. Periods of activity of *Myotis l. lucifugus* in normal cycle and in continuous darkness, to illustrate a 24-hour rhythm of activity of bats which persists in darkness. After activity the body temperature of this animal probably decreased. Its biological clock would have to be temperature compensated. From Griffin and Welsh (1937).

Discussion

There is a regular pattern of awakening from hibernation in ground squirrels, but there is no clue in the study of heart rates as to a persistent regularity in physiological function during deep hibernation. The body temperature under these circumstances (5°C) may be too low (there are no other animal rhythms at this temperature). Either the changes in approximately 12-hour units during hibernation are not reflected in heart beat or else there is a relatively steady state in all functions. At the moment, the most plausible theory for the regular awakening of

ground squirrels from hibernation appears to be as follows: the animals start to come out of dormancy; if they are in a period of darkness, then progress is slow but if they have a dim light shining on the cage, then the awakening from dormancy is more rapid. Some of the slow-awakeners will delay their progress until the light period. Few of those in the light will postpone their awakening until the dark period occurs.

The regular day-night activity of cooled lizards and bats still must be explained. It is apparent that animals other than mammals correct for the effects of cooling, and some cooled mammals show normal activity of nerve tissue within at least the upper part of the body temperature range. There must be enzymatic adjustments which permit this cold compensation. Some theoretical considerations associated with this hypothetical enzymatic adjustment are now presented. Attempts to explain the temperature independence of biological rhythms cannot be separated from the search for the nature and location of the biological clock. This "structure" can be visualized as either a localized area or a diffuse collection involving many types of tissues. It is of interest that lack of agreement has occurred previously concerning the nature of the causes initiating some specific physiological processes. A localized origin was postulated by Cannon (1918) to explain thirst in mammals and by Lorand (1912) to explain the aging process in vertebrates. Others now believe in diffuse sources to explain both of these phenomena. In the present case of the biological clock we must look not only for this clock but for a "structure" which by definition also has a very unique biological characteristic, that of temperature independence. This characteristic is so unusual that our credulity is less tried by supposing a discrete localized clock which has a special mechanism to permit performance at low temperatures. If we assume the alternative (a diffuse series of clocks in each animal) there must be a broader distribution than we have supposed of the rare biological material which can display temperature independence. The clock-identification is complicated, as usual, by the fact that one-celled animals have temperature-independent endogenous rhythms.

Some scanty evidence exists of two types of physiological mechanisms which increase their activity as temperature is lowered, including (1) enzymatic activity, and (2) action potentials of brain tissue (EEG). There is not direct evidence of enzymes which increase their activity as temperature is lowered,

but Precht (1958) has shown that some oxidative enzymes of fish show a decrease in activity after acclimatization to a raised temperature. Presumably these enzymes also show the opposite: an increase in enzymatic activity when temperature is lowered. A clue to this behavior is given by Swartz *et al.* (1956) who studied enzymes which were activated by heat. This occurred due to the fortuitous coincidence of a heat-stable enzyme and a heat-labile inhibitor. In a similar fashion a temperature-independent biological clock needs only to possess a collection of cold-stable enzymes and cold-labile inhibitors which release more of the enzymes as the temperature is lowered. Bunning and Leimweber (1956) provide a slightly different theory: "It is by no means impossible to imagine a chemical mechanism that also has no temperature dependence. If part of the mechanism were concerned with supplying a particular substance while a second process destroyed it and both processes were equally temperature dependent, then the substance would accumulate at the same rate irrespective of temperature." Perhaps such enzymes are involved in the observation of Suda *et al.* (1956) who showed that the EEG of the cat shows an *increase* in amplitude as temperature is lowered from 37°C to 24°C.

Other widely different approaches to explaining temperature independence remain to be discussed. The first is described as part of Pittendrigh and Bruce's (1957) oscillation model of the basic biological rhythm in any animal. They refer to this as an endogenous self-sustaining oscillation (ESSO), but believe that the control or clock presiding over this oscillation is a complex system with constituent oscillatory processes. The control system is not a single temperature insensitive process. Then they explain, "The mutual entrainment (synchronization) of constituent oscillators would result in temperature independence over a limited range provided that key members of the system had reciprocal temperature coefficients." They note further that temperature has been used to replace light for reversing rhythms by 12 hours. If this is so, it is difficult to conceive of a process (the clock) which *responds* to temperature but is also insensitive to temperature. Pittendrigh and Bruce's argument supports their description of the clock as a *system* of compensating processes (some of which respond to temperature, while others being resistant to temperature, can restore synchronization).

The second and final approach to explaining the temperature independence of persisting rhythms concerns the possible response by cold animals to subtle environmental clues. As Brown

(1957) expresses it: "The main difficulty is to explain how a metabolic clock can maintain such uncanny precision over a temperature range of more than 20 degrees C. An alternative hypothesis which fits all the known facts equally well is that the mechanism is one which can perceive some kind of physical force in the environment hitherto not known to affect living organisms." If any physical time-giver other than light affects the cold animal in darkness, there is no reason to suppose it cannot respond to this, since the cold animal still responds to a light cycle. (The rhythm of a cold animal can be reversed by 12 hours of light.) One particular merit to the hypothesis of Brown is that it does not require that any tissue or tissues of the body behave biochemically in an atypical fashion.

To summarize: we have been considering what mechanisms could be responsible for the regular rhythm of activity in animals like the lizard and bat in darkness, when their blood is cool or cold. One theory includes a chemical mechanism without temperature-dependence, another depends upon a system of constituent oscillators, and the third refers to the possibility of subtle environmental clues. This theorizing at the moment should be applied only to bats and probably ground squirrels at moderate temperatures (15-18°C) where they both show a drop in body temperature. Although ground squirrels may *awaken* from deep hibernation as if influenced by a 24-hour rhythm, no physiological evidence of this rhythm was found with ground squirrels *in deep hibernation;* likewise, little evidence was found in hibernating bats. Future experiments should be done with hibernation at a warmer temperature (10°C) and without a light cycle in the cold chamber. The regularity of awakening from deep hibernation by ground squirrels is best explained as due to possible speeding of the awakening process by the environmental clue of light. An alternative theory is less plausible until some evidence is obtained of a physiological rhythm in deep hibernation.

Acknowledgments

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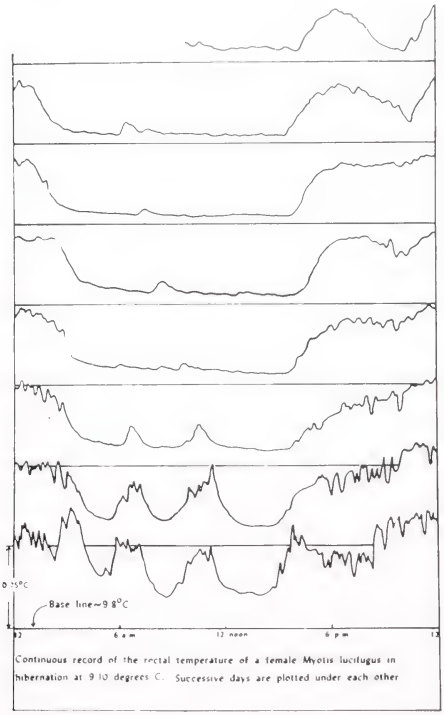


Continuous record of the rectal temperature of a female Myotis lucifugus in hibernation at 9-10 degrees C. Successive days are plotted under each other

Fig. 14

DISCUSSION FOLLOWING FOLK'S PAPER

MENAKER showed a slide (Fig. 14) indicating that rhythmic fluctuations in body temperature persist in the little brown bat.

Myotis lucifugus, down to body temperatures of 10°C in constant darkness without awakening of the animal. He cited this as evidence that the "clock" of bats continues to function at this low temperature.

SOUTH indicated that in studying "clock" mechanisms in rats almost any cue would reset a "biological clock," for example, a hole in a chamber admitting a small amount of light. FOLK answered by stating that it is his belief that an animal hibernating or in an essentially stable environment is in a sense "hungry for cues" to assist its own "clock" mechanism. SOUTH asked if a 25½ hour cue could be used to "confuse the clock." FOLK replied that Pittendrigh reports that a range of cues given between 22-27 hours could be followed, but outside of this range the animal would resist resetting its "clock" mechanism (C. S. Pittendrigh in *Symposium on Perspectives in Marine Biology*, Berkeley, 1957, p. 255).

XII

BROWN FAT AND ITS POSSIBLE SIGNIFICANCE FOR HIBERNATION

By Bengt Johansson
Cardiological Laboratory, Department of Medicine
Malmö General Hospital
Malmö, Sweden

Generally, the term "fat" is used to designate the yellowish or white fat occurring mainly in the subcutaneous fat depots. There is, however, another type of fat; one of its names is "brown fat." It is said to have been described first by Velsch (see Auerbach, 1901-02) in 1670 or perhaps by Gressner (see Bole *et al.*, 1951) as early as 1551. In spite of this, it took many years before brown fat was generally considered as a separate entity; it was often mistaken for thymus tissue. At the turn of the century, however, excellent gross and microscopic descriptions of brown fat appeared such as those by Hammar (1895) and Auerbach (1901-02). Later more detailed studies, especially on metabolic aspects and physiological function, have appeared. It is the aim of this paper to give a short summary of our knowledge about brown fat and to discuss, especially, two things:

(1) Are the differences between brown and white fatty tissue anatomically and functionally so great as to allow a definite separation between these two tissues?

(2) How valid is the evidence of a connection between brown fat and natural hibernation in mammals, evidences which have created such synonyms for brown fat as "hibernating gland," "masse hibernale," and "Winterschlafdrüse" or "Winterschlaforgan"?

Gross and Microscopic Appearance

In the animals investigated, including both hibernators and non-hibernators (Rasmussen, 1923; Wegener, 1951), brown fat has been found in essentially the same places in all species.

Hammar (1895) has given a detailed report of the appearance of brown fat in rats. In the thorax, the brown fat is situated as a strip on the ventral part of the spine surrounding the descending part of the aorta. This dorsal mass proceeds ventrally through the mediastinum towards the dorsal side of the sternum where it forms two strips with small extensions on the costal

cartilages. The brown fat which is situated on the vertebrae continues into the abdomen, always surrounding the aorta and the inferior vena cava. At the aortic bifurcation one part follows the external iliac vessels ending just above the inguinal ligament; another smaller part may follow the internal iliac vessels. Sometimes a small amount is found in the groin. Brown fat is most abundant in the back, especially interscapularly, extending between the muscles ventrally and laterally. The axillae can give accommodation for rather large amounts of brown fat which is also found in the ventral part of the neck — here, however, to a much lesser extent. Thus the brown fat is mostly situated in the inner part of the body, while most of the white fat is situated more peripherally (Feyrter, 1945-48).

The microscopical picture of brown fat differs principally from that of white fat in having smaller cells with a round nucleus, and multiple fat droplets within the cell in contrast to the bigger white fat cell with a flattened peripheral nucleus and a single big fat droplet. Furthermore, the cytoplasm in brown fat is much more abundant than in white fat. Lachance (1953) points out that brown fat contains 15 per cent non-fatty dry material and 46 per cent water. Corresponding figures in white fat are 1 and 11 per cent. The lipids, however, are more abundant in white fat — 88 per cent, against 39 per cent found in brown fat. Schierer's (1956) value for non-fatty dry material in white fatty tissue in the hamster is somewhat higher, 3.3 per cent, while the value found in brown fat, 14.9 per cent, is very close to that reported by Lachance (1953).

In the rat, a non-hibernator, the brown fat is arranged in lobules and these are separated by connective tissue, into which the blood vessels extend (Hammar, 1895). The brown fat cells are polygonal and contain many fat droplets all of about the same size. The cytoplasm is rather coarsely granular. One, sometimes two, nuclei are found, most often situated somewhat eccentrically but not as far toward the periphery as in white fat. The vascular supply is rich. The black and pale grey color after injection of a black dye into brown and white fat, respectively, indicates that the blood supply is more ample in the brown than in the white fatty tissue.

In extreme emaciation of the rat, the brown fat has a dark reddish-brown color. The vascular content is abundant with dilated vessels. In some cells a few small fat droplets remain. On the other hand, in fattened rats the color is pale whitish-brown but still stands out distinctly against the white fat. The

fat droplets are bigger but there is no essential difference from the picture found in a medium nourished animal. In the periphery of the lobules, however, the fat droplets may sometimes fuse to form one single drop.

In the woodchuck, a hibernator, the appearance of brown fat differs in certain respects from that found in the rat although the essential characteristics remain (Rasmussen, 1923). The cell contains one and occasionally two large fat globules and many smaller ones of various sizes. Because of this, the nucleus is more eccentric than in the rat. However, it is never flattened much, if at all, no matter how fat the animal is. The amount of brown fat shows a decrease during hibernation accompanied by an increase in the intensity of the brown color. The fat droplets disappear but only to a certain extent. The brown fat in man has recently been described by Wegener (1951). This author points out the varying size of the cells and of the fat vacuoles of different cells. No essential differences are, however, found microscopically in human brown fat compared with the above-mentioned forms.

Biochemical Observations in Brown Fat

Different components of brown fat have been investigated with chemical analyses and also microscopically with histochemical methods. (See Johansson, 1959, for a review of the literature.)

The carbohydrate content has been found to be higher in brown fat than in white, at re-feeding after starvation. The lipids, however, are less abundant in brown fat. Significant differences in iodine number have not been found (Shattock, 1909; Coninx-Girardet, 1927) although histochemical methods indicate that the lipids in brown fat are more saturated than in white. During hibernation, the weight and lipid content decrease and the amount of water shows an increase. According to Carlier and Evans (1903) the lipid decrease in brown fat from hedgehogs begins rather late during hibernation (in the month of January). After injection of labeled phosphorus in milk to rats, the specific activity has been found to be eight times less in white than in brown fatty tissue where the activity is the same as in gland tissue (Littrell *et al.*, 1944; Favarger and Gerlach, 1955). The concentration of different enzymes has been determined. As for glycogenesis, Shapiro and Wertheimer (1956) claim that all enzymes necessary for this process occur in brown fat. The

chemical properties of hexokinase, phosphoglucomutase and phosphorylase from brown fat resemble those found in muscle (Creasy and Gray, 1952).

Fawcett (1952) has made a comparison of the cytochemical reactions of brown and white adipose tissues in rats and mice. Both brown and white fatty tissue contain neutral fats; in the former, however, these are more saturated and less abundant. The cytoplasm of brown fat appears richer in phospholipids, acetyl phospholipids and glycogen. Both tissues contain esterase, succinic dehydrogenase and alkaline phosphatase, but they seem to be more ample in brown fat. Cholesterol and ascorbic acid were found neither in brown nor in white fat. Menschik (1953) has performed a similar histochemical study in guinea-pigs. In contrast to Fawcett (1952) he found a conspicuous amount of succinic dehydrogenase. Furthermore, Menschik (1953) claims that brown fatty tissue contains more α-amino acid groups, mucoproteins, watersoluble polysaccharides, glucolipids, phospholipids, cholesterol and its esters, amine oxidase, α-naphthol oxidase and cytochrome oxidase but less neutral fat than white fatty tissue. Rémillard (1958) has recently presented a detailed paper on the histochemistry of brown fat in a bat.

When measured by the Warburg technique the oxygen consumption has been found to be higher in brown fat than in white. It shows a seasonal variation, being highest around September and October. When using a substrate like pyruvate or succinate, no difference in oxygen consumption of brown fat was obtained between hibernating and non-hibernating animals at the same temperature. In contrast, the oxygen consumption in kidney slices was diminished by 12 per cent in the hibernating animal (Hook and Barron, 1941).

The cause of the brown color has not been satisfactorily explained. The rich vascularization probably contributes to it in part. Seasonal variations also play a role, for during hibernation the brown fat becomes darker. The brown color has, among other things, been ascribed to oxidation products of phospholipids, to hemoglobin, hemosiderin, hemines and lipochrome.

Influence of Various Hormones

ACTH produces an increase of water, lipids and fat-free dry material, and the fat droplets fuse to form a large single drop, the cells assuming the character of white fat.

Brown fat takes part in the reaction to stress (Lemonde and Timiras, 1951). There is a close parallelism between the reactions of the adrenal cortex and of the brown fatty tissue. Suomalainen and Herlevi (1951) have studied the brown fat during the arousal process of hibernating animals and conclude that the arousal represents a stress to the animal.

Injections of adrenocortical hormones have given somewhat different results. These are, partly at least, caused by inconsistency in dose, length of observation time and age of the animals. Aronson *et al.* (1954) describe a hypertrophy of the brown interscapular fat after cortisone injection, more pronounced in hamsters than in mice. Lachance (1953) has found an increase of the lipid content after cortisone; cortisone exerts a greater influence upon the weight of the interscapular body than desoxycorticosterone in adrenalectomized rats.

Thyroxine injected into rats results in hypertrophy of the interscapular brown fat; it is due to an increase in the lipids, whereas water and fat-free dry material remain unchanged (Lachance, 1953). Thiouracil treatment splits up the fat droplets so that they decrease in size and increase in number (Littrell, 1948; Fawcett and Jones, 1949).

After starvation and refeeding, glycogen can be demonstrated in both brown and white fatty tissue, comparatively more, however, in the former. Insulin causes a deposition of glycogen in both forms of fat.

Discussion

Earlier authors have discussed the importance of brown fat for hibernation and many have stressed a causal relationship. Recently, however, Herter (1956) and Schierer (1956) have emphatically denied such a connection, and other authors have refuted a definite position.

To solve this problem it is necessary to have an answer to at least three questions:

1) Are the differences between brown and white fat great enough to characterize them as two different tissues?

2) Does brown fat exist in all hibernators?

3) How valid are those experiments showing a causal relationship between brown fat and hibernation?

1) Schierer (1956) vigorously claims that brown fat is only a fat deposit, that the biochemical differences between the two sorts of fat are all due to the greater amount of cytoplasm in

the brown fat, and that brown fat has no connection with hibernation. In her experiments she has used wild rats and hamsters but the number of animals is very small.

The histochemical studies by Fawcett (1952) and Menschik (1953) were unfortunately performed only on non-hibernators. Schierer (1956) has compared rats and hamsters histochemically and found no differences between these two animals. Histochemical studies are often more qualitative than quantitative, and, although Menschik (1953) tries to take into consideration the different amount of cytoplasm, it is possible that many of the differences in amount found by these two authors are not real. Mirski (1942), however, using the ability of converting fructose-1-phosphate (Cori ester) to fructose-6-phosphate (Robison ester) as a test of the phosphoglucomutase content, claims that white fat lacks this enzyme in contradistinction to brown fat.

The O_2-consumption is much higher for brown than white fat; judging by the figures given by Hook and Barron (1941) it seems as if the difference is greater than can be explained by the different amounts of cytoplasm. It has also been shown that brown fat retains a comparatively high metabolic activity during hibernation as reflected in oxygen consumption (Hook and Barron, 1941) and redox potentials (Klar, 1941). Many authors have stressed the glandlike appearance of brown fatty tissue in contrast to white. Schierer (1956), too, admits that the cells of brown fat have an epithelioid arrangement and that the vascular supply is abundant. These properties are found in endocrine glands also. She points out, however, that brown fat lacks certain essential qualities characteristic of endocrine glands.

Some important facts have not been considered by Schierer (1956) — for example, different reactions of brown and white fatty tissue to various hormones and vitamins. Cortisone causes a hypertrophy of brown fat (Aronson *et al.*, 1954) as does thyroxine (Lachance, 1953), while white fat shows no change and a decrease, respectively. The response to stress is much more pronounced in the brown fat (Selye and Timiras, 1949). So is the response to hypophysectomy, adrenalectomy and thiouracil feeding (Fawcett and Jones, 1949). Vitamin E deficiency causes pigmentation and cellular developmental changes in white fat, while brown fat remains unchanged (Mason *et al.*, 1946). The amount of androgens is said to be very high (Sweet and Hoskins, 1940), and hormones of the corticosteroid type have been described in brown fat (Nigeon-Dureuil *et al.*, 1955; Ratsimamanga

et al., 1958; Zizine, 1958). Certain types of virus show predilection for brown fat (Pappenheimer *et al.*, 1950; Godman *et al.*, 1952; Aronson and Shwartzman, 1956; Sulkin *et al.*, 1957). In starvation there are nearly always some fat droplets left in brown fatty tissue, which retains its characteristic aspects, while the white fat shows a total depletion and adopts the character of fibrocytes. In fattening, some of the brown fat cells show a fusion of the fat droplets. This, however, occurs only in the periphery; in the central parts the cells retain their characteristic appearance. According to Hammar (1895), brown fatty tissue shows an embryological development different from that of white fatty tissue and the blood supply is richer. Fawcett (1952) states that white fat can be found in all places where connective tissue abounds while the distribution of brown fat is confined to certain definite localities in the body. It seems, then, that brown fatty tissue possesses so many properties differing from white that they can be looked upon as two different tissues. This, of course, does not exclude the fact that brown fat can act as a fat deposit, too. From a teleological point of view, however, it is remarkable that it contains so much cytoplasm if its only task is to store lipids.

2) If brown fat has a close connection with hibernation, it should be found in all hibernators. There seems to be a general agreement among workers in this field that brown fat is found in all hibernators.[1] In Rasmussen's (1923) review he mentions two "hibernators" that lack brown fat, the badger and the raccoon. The badger, however, is no real hibernator. During the winter it enters a state of winter torpor, but like the bear (Hock, 1958) the animals are not poikilothermic. Personal investigations on the badger show that this animal during the winter does not behave biochemically in the same way as a real hibernator like the hedgehog, and, most important of all, when being cooled the heart stops beating at about +13°C; the hedgehog's heart still beats a few degrees above zero (Johansson, 1957a). Cushing and Goetsch (1915), in a footnote, characterize the raccoon as a hibernator without brown fat. Later authors, however, are of the opinion that this animal is not a hibernator (Eisentraut, 1956).

As stated earlier, brown fat is found not only in all hibernators but also in many non-hibernators. This has been taken as an

[1] In response to my query, Drs. Eisentraut, Herter, Kayser and Lyman all assure me that they know of no hibernators which do not have brown fat.

argument against a connection between brown fat and hibernation. There are, however, examples in the realm of animals that an organ remains although its original task has disappeared or changed. It seems as if no extensive investigation has been performed to find out whether hibernators contain more brown fat than non-hibernators. Preliminary personal investigations indicate that hibernators like the hedgehog have definitely more brown fat than non-hibernators and that among non-hibernators the rat contains more brown fat than, for example, the guinea-pig. The brown color in rats seems to be less pronounced than in hedgehogs.

There is a tendency for the hearts of rats at least to withstand cooling better than, for example, guinea-pigs which have less brown fatty tissue. Furthermore, rats and mice have an extremely short S-T interval in the electrocardiogram, which has been found to be typical of many hibernators (Johansson, 1957b).

3) There are at least two different ways of studying the function of brown fat. One way is to extirpate or destroy the brown fat, another to extract the possible active substances.

Extirpation experiments are very difficult because brown fat is so widespread that it is impossible to perform a complete removal. The results of such experiments have been divergent.

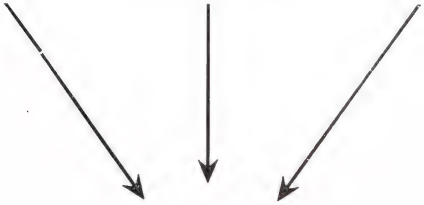
Kayser (1953) could not find any differences in the resistance of rats and hamsters to cold after extirpation of the "hibernating gland." Vignes (1913) states that removal of the brown fat in rats causes a decrease of the body weight and the animals gradually die. Trusler *et al.* (1953) conclude that in marmots extirpation of the brown fat causes a decrease of the resistance to cooling. Zirm (1956a) has found that hedgehogs die upon exposure to extreme cold during hibernation after extirpation of about 50 per cent of the total amount of brown fat.

Wendt (1943) has published the results of experiments with an extract from brown fat which was injected *inter alia* into rats. He found that such injections depress the basal metabolic rate, pulse rate, blood pressure and body temperature. Recently Zirm (1956a,b) has shown that implantation of pieces of tissue from the "hibernating gland" of hibernating hedgehogs into mice causes a decrease of the body temperature of the animals in proportion to the size of the implant. In addition, within two weeks of the implantation the animals increased in body weight. Zirm also succeeded in preparing a yellow-green

substance from the "hibernating gland." Injection of this preparation into mice was followed by a drop in the temperature, respiratory frequency and blood pressure. Extracts from brown fat from non-hibernating hedgehogs and extracts prepared in the same way from the liver, lungs, spleen or kidneys of hibernating or non-hibernating hedgehogs produce no such effect.

TABLE I

Exogenous factors	*Endogenous factors*	*Inborn qualities*
1. Temperature	1. Inhibition of temperature regulation.	Ability of the tissues (especially the cardiovascular and the nervous systems) to function at temperatures just above 0°C.
2. Rest	2. Increase of fat depots.	
3. Light	3. Hypertrophy of brown fat.	
4. Deprival	4. Polyendocrine involution.	
5. Composition of food (especially decrease of water content).	5. Decrease of sympathetic and increase of parasympathetic tone.	
6. Confined air.		



HIBERNATION

The importance for hibernation of experiments with extracts of brown fat has been denied by Wertheimer and Shapiro (1948), among others, who believe that the retarding effect of brown fat on metabolism is non-specific.

There is one point in connection with these extraction experiments that I should like to point out. As is shown in Table I, it is not only the exogenous factors and the endogenous change in the autumn that are necessary for the entrance of hibernation. The organism must also be able to maintain at least some circulation during hibernation, i.e. the heart must be able to beat at the low temperatures that obtain during hibernation. This is a property that is not restricted to the hibernation period but one which the hibernators show during the whole year and which

adult non-hibernators never show. Preliminary experiments on a small number of rats indicate that in animals given a diet with a high percentage of corn oil, containing a high amount of unsaturated fatty acids, the hearts keep beating at a lower temperature than in rats given coconut oil which contains a high percentage of saturated fatty acids. It is thus possible to change the heart's resistance to cooling by exogenous means, but I think this is rather far from stating that a single injection of some material, as has been done in the extraction experiments, could give a non-hibernator's heart the ability of performing work at a temperature just above zero. The conclusion is, then, that the extracts of brown fat should be tested also on hibernators in a non-hibernating state to get a real appraisal of the ability of the extracts to induce hibernation. The problem of how hibernators can stand very low temperatures must, I think, be solved by comparative biochemical studies, especially a comparison of the properties of different enzyme systems. Studies of this type have just started at the Malmö General Hospital.

From the above it is apparent that no valid objections have been given against a connection between brown fat and hibernation. On the other hand, although some interesting facts point in this direction, no definite evidence has been given stating that there really is such a connection. It seems to me that the experiments that are most interesting and that in the future will contribute the most conclusive evidence to the solution of this problem are of the type that have been performed by Wendt (1943), Hook (1940) and recently, by Zirm (1956b).

Summary

A short review of some facts on brown fat is given. The author discusses the possible connection between brown fat and hibernation. It is concluded that brown fat occurs in all hibernators and that the occurrence of brown fat in many non-hibernators must not be considered evidence against a possible connection between brown fat and hibernation, for there are examples that an organ can remain even after having lost its original task. The differences existing between brown and white fatty tissue are considered so great that there are reasons to look upon them as different tissues. This does not exclude the fact that brown fatty tissue can also act as a depot for lipids. Different ways to show experimentally a connection between brown fat and hibernation are discussed. Experiments with extracts

of brown fat seem to give the most interesting results. The necessity of using the extracts on hibernators in a non-hibernating state in order to induce hibernation is stressed.

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DISCUSSION FOLLOWING JOHANSSON'S PAPER

SMITH reported that he had removed substantially all the brown fat from bats and had discovered that such animals (less brown fat) also lowered their body temperatures in the face of a cold stress. JOHANSSON indicated that all brown fat could not be removed by gross surgery, but if the brown fat got its blood supply from one or a few main vessels, ligation of these might cause a complete loss of the brown fat from the entire body.

PEARSON said he had carried out brown fat injection experiments using the interscapular gland of *Myotis*, and found that this tissue was very toxic and would kill white mice when injected.

JOHANSSON believed it should be established whether brown fat, taken from animals in the hibernating state and injected into animals of the same species during the non-hibernating season, makes it easier for such animals to enter hibernation.

MORRISON pointed out that there may be difficulty in studying these effects in an animal (white mouse) not constitutionally arranged for hibernation. He noted that his group tried without success to lower the body temperature of white mice by implants or injection of brown fat homogenates from hibernating 13-lined ground squirrels.

KAYSER said the best experiments to this end have been done by G. A. Trusler *et al.* (Surgical Forum of 1953, **4**:72, 1954). The body temperature at which respiration will cease is a lower temperature when sufficient brown fat is present, than when it is in short supply. He indicated, however, that he had not been able to repeat Zirm's experiments with the hedgehog; he felt he may have destroyed the principle in preparation. On the other hand,

he believed that hypothermia depended on the amount of brown fat. He indicated that he has totally reversed his former opinion, and he now believes brown fat is important in hypothermia and hibernation.

JOHANSSON asked HOCK if brown fat was found in bears or badgers, although these animals are not true hibernators. HOCK indicated bears have some, but of an unimportant quantity comparatively. JOHANSSON added that brown fat is present in a comparatively large amount in small children.

ZIMNY called attention to the study of brown fat reported by G. Rémillard (Ann. N. Y. Acad. Sci., **72**:1, 1958).

BALL remarked that in the rat the brown color of the fat seems to be entirely due to the high content of cytochromes which presumably act as an energy supply. With reference to cytochromes and tissue metabolism, ZIMNY indicated that biochemical studies of glycogen, lactate and pyruvate showed variations in these compounds during hibernation.

XIII

SOME PROBLEMS OF REPRODUCTION IN RELATION TO HIBERNATION IN BATS[1]

By William A. Wimsatt
Department of Zoology
Cornell University
Ithaca, New York

Introduction

Many physiological aspects of hibernation are no doubt common to all mammalian hibernators, but there are a few which are peculiar to individual species or groups. My purpose here is to review one of these less generalized phenomena, the profound influence of hibernation on the physiology of reproduction in bats. I intend not so much to provide answers, few of which have as yet been forthcoming, as to indicate the nature of some of the more important problems still unsolved.

It is characteristic of nearly all hibernating mammals that the annual periods of hibernation and reproduction do not overlap, at least not significantly, and it is doubtful that the profound metabolic depression of hibernation has any important influence on reproductive activity, except possibly to delay its onset. These species, which include all the more familiar hibernators such as the woodchuck (*Marmota*), hedgehog (*Erinaceus*) and ground squirrels (*Citellus*), are typically spring breeders. They enter, and pass through hibernation in a state of virtual sexual quiescence, or anestrum, and both males and females become sexually active only after awakening and emerging from hibernation in the spring. There appears no reason to suppose that the factors which condition reproductive activity in these species are qualitatively any different from those operative in other spring-breeding mammals which do not hibernate.

By contrast, it is now widely recognized that hibernating bats present a unique departure from this general pattern, for in them hibernation falls fairly astride the reproductive season, and influences to a marked degree the reproductive physiology of both sexes. It should of course be emphasized that not all bats hibernate, or even necessarily possess the capacity to do so.

[1] The original work reported herein was supported by research grants (G-2188 and G-7474) from the National Science Foundation.

Bats are tropical in origin and primary distribution, and annually recurring periods of hibernation are observed in only the few species (belonging to but two families, Vespertilionidae and Rhinolophidae) which have become adapted to living in the cooler temperate latitudes. Significantly, these alone manifest the reproductive peculiarities for which bats are noted (cf. reviews of Wimsatt and Trapido, 1952, and Herlant, 1953).

Background: The Reproductive Cycles of Hibernating Bats

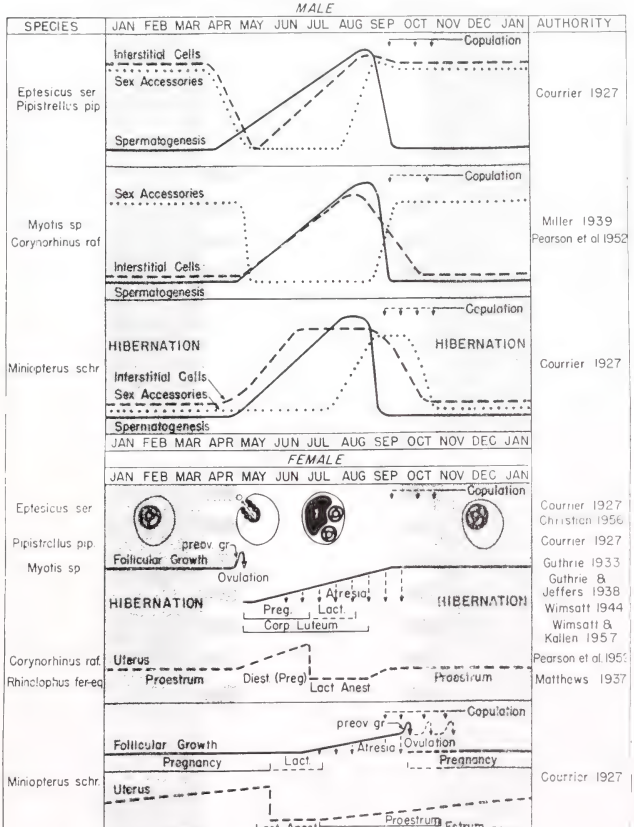
The chronology of major reproductive events in relation to the annual cycle of hibernation in bats is summarized schematically in Figure 1. Differences between species occur in the timing and degree of expression of some of the phenomena shown, but at least as far as the female is concerned the basic picture is similar in all hibernating bats, with the single exception of the European vespertilionid, *Miniopterus schreibersii*, in which the cycle is depicted separately. In contrast to other hibernating mammals, bats initiate reproductive activity in the fall, but typically enter hibernation very soon thereafter. Dormancy appears to arrest the normal progress of reproductive events, and postpones their completion until after the animals permanently emerge from hibernation in the spring.

The reproductive status of males and females before, during and after hibernation may be summarized as follows. In males the spermiogenic phase (of spermatogenesis) is achieved in late summer. When copulations begin, just prior to hibernation, the cauda epididymides are congested with mature spermatozoa, which continue to reside here throughout the period of dormancy. However, the spermatogenic activity of the testis has by now spent itself, and at the time of the fall copulations the seminiferous tubules are rapidly reverting to the quiescent state characteristic of the hibernating period, consisting in the main only of spermatogonia and Sertoli cells. The sex accessory glands remain small and unstimulated throughout the summer spermatogenic phase, but suddenly and rapidly hypertrophy over a short interval in the fall which coincides with pairing and copulation. When the males enter hibernation soon after copulating the sex accessories are still in a maximally hypertrophied state, and they remain more or less enlarged throughout the period of dormancy. Involution occurs suddenly, however, following emergence from hibernation in the spring (Courrier, 1927; Miller, 1939; Pearson *et al.*, 1952; Krutsch, 1956; personal

observations). In the foregoing respects the cycles of all male hibernating bats except *Miniopterus* are complementary; the latter differs in that the sexual accessories involute completely during the fall. There is an apparent paradox, however, in respect to the changes in the interstitial cells in various species.

FIGURE 1

REPRODUCTIVE CYCLES IN HIBERNATING BATS



According to Courrier (1927), in the European *Pipistrellus* and *Eptesicus* the Leydig cells are maximally developed at summer's end when the sex accessory glands achieve maximum development, but regress somewhat before the males enter hibernation, and then experience profound involution at hibernation's end in the spring. In closely related American species, on the other hand (*Myotis*, Miller, 1939; *Corynorhinus*, Pearson *et al.*, 1952; *Pipistrellus*, Krutsch, 1956), the interstitial cells are involuting during the time that the sex accessories are enlarging, and appear most active in summer, when the accessories are small and show no evidence of endocrine stimulation. In *Corynorhinus*, at least, they are also large in sexually immature males. The histological picture in the European bats is consistent with the view that the interstitial cells produce the male hormone, but the situation in the American species is more difficult to reconcile with this concept. It is perhaps an anticlimax to record that in many species, the males, when temporarily awake during hibernation, demonstrate the continuing capacity to copulate throughout this period (Guthrie, 1933; Wimsatt, 1944a; Guilday, 1948; Pearson *et al.*, 1952). Summarizing, the cycle in the male bat is peculiar in at least three respects: 1) there is an apparent exaggerated asynchrony between the spermatogenic and endocrine cycles of the testis; 2) viable spermatozoa persist in the epididymis for an unusually prolonged period after the cessation of spermatogenesis; and 3) the apparent functional cycle of the Leydig cells of the testes, as judged by the usual histological criteria, does not coincide completely with the periods of libido and functional hypertrophy of the sex accessory glands in American species, but appears to do so in some European ones.

The situation in the female is more constant, but no less striking. All known species of hibernating bats are monestrous, but there is no true period of anestrum. In adults a new estrous cycle is inaugurated shortly after lactation ends in late summer, and growth and atresia of vesicular follicles occurs in most species during the weeks between lactation's end and entrance into hibernation. Copulation occurs near the end of this period, at which time the number of vesicular follicles in the ovaries has been reduced, and the animals enter hibernation with one or more follicles (species differences) destined to survive throughout the winter. Young females of some species are inseminated in their first fall, but copulation in these instances usually occurs in the absence of vesicular follicles in the ovaries (Guthrie, 1933;

Wimsatt, 1944b). At the time of the fall inseminations and entrance into hibernation, the oviducts, uterus and vagina show all the characteristics of proestrous stimulation, a condition which they maintain throughout the hibernating period. Spermatozoa from the fall and later inseminations are stored either in the uterus in the Vespertilionidae, or in the vagina in the Rhinolophidae (Matthews, 1937), and it has been demonstrated that they remain viable throughout the period of dormancy (Wimsatt, 1942; 1944a). The surviving large follicle, which develops a unique structural and chemical organization, remains unchanged throughout hibernation (Wimsatt, 1944b; Wimsatt and Kallen, 1957). Preovulatory growth and rupture of the surviving follicles occur in the spring, within a few days after the females leave hibernation (Wimsatt, 1944b; Sluiter and Bels, 1951). Summarizing, the unusual features of reproduction in the female bat are: 1) the prolongation of the proestrous phase of the cycle through hibernation, 2) the maintenance of viable spermatozoa in the reproductive tract throughout the period of dormancy, and 3) the marked delay in ovulation involving the prolonged survival through hibernation of the follicles destined to rupture in the spring.

As mentioned earlier, the only known exception to this general picture in hibernating bats is the vespertilionid bat *Miniopterus schreibersii*. As shown in Figure 1, there is no delayed ovulation; follicular rupture follows soon after the fall copulations, and pregnancy ensues immediately. Embryonic development is alleged to be retarded, but not actually arrested during hibernation, and the young are born some time after hibernation ends, in the early summer (Courrier, 1927). The gestation period is some months longer than in tropical members of the same genus, and paradoxically, the breeding season is reversed. In the tropics the Miniopterinae breed at a time of year which corresponds to the northern spring (Baker and Bird, 1936), whereas the European species breeds in the fall.

Nature of the Problem

Up to the present day attention has been focused primarily on the chronological and morphological aspects of reproduction in hibernating bats, together with attempts to deduce on an analogical basis the underlying endocrine mechanisms. Experimental analyses have been sporadically undertaken, mostly in respect to the cycle in the female, but for the most part they have been

limited in scope, and have contributed little toward the formulation of an integrated picture of the controlling factors of reproduction such as we possess for other mammals. Likewise, scant attention has been paid to the immediate physiological bearing of dormancy *per se* on the mode of action of the agencies controlling reproductive phenomena in bats, and indeed there seems to have been little appreciation that the latter might be of any significance! For these reasons it seems worthwhile to outline a few of the more important gaps in our knowledge of these matters in male and female bats, and to review recent experiments of my own and others which relate to some of them.

The greatest deficiencies are found in our knowledge of the intrinsic endocrine and neural mechanisms which in bats as in other mammals presumably regulate reproductive processes, and the possible ways in which hibernation directly or indirectly modifies their action. We are also essentially ignorant of the possible influence of external environmental stimuli on regulation of reproductive periodicity in bats, and of the sensory pathways through which their effects might be mediated.

The male bat. I shall begin with the male which, though less studied from an experimental point of view than the female, seems from the timing of its cycle to present fewer problems, and can therefore be more rapidly dealt with.

Possibly a significant problem for investigation exists in those American bats (e.g. *Corynorhinus*) in which libido, viable epididymal spermatozoa, and hypertrophied sex accessories, are established and maintained over a long period during which the interstitial cells of the testis appear to be in a functionally involuted state. If one assumes that these phenomena in bats are conditioned by testosterone as in other mammals, he must conclude that in these American species, in contrast to the European ones described by Courrier, the usual histological criteria are of little value in assessing interstitial cell function, and that an apparent involuted condition of the interstitial tissue is commensurate with a maximal hormone release. If this proves correct, then we are still faced with the problem of reinterpreting the different conditions in Courrier's bats. The picture in the American bats is perhaps further complicated by the fact that an attempt by Pearson *et al.* (1952) to demonstrate steroidal compounds in the interstitial tissue of hibernating specimens of *Corynorhinus* by a histochemical test (2-4 dinitrophenylhydrazine reaction) failed to reveal any, in either interstitial tissue or

tubules, and under the same conditions of testing in which control mouse testes reacted admirably. A more thorough histochemical and biochemical study of the androgen content of the testis during hibernation is obviously indicated, for it has been demonstrated by both Courrier (1927) and Pearson *et al.* (1952) that the sex accessories of male bats are indeed responsive to androgen, at least in active animals (*vide infra*).

There is an alternative possibility which to my mind has neither been adequately considered nor effectively ruled out. It is the possibility that the testes no longer actively produce hormone during hibernation, but that torpidity itself retards the regressive changes which at more elevated body temperatures quickly follow hormone withdrawal. There is some suggestive evidence in support of this hypothesis, and some which militates against it. In support, are the observations of Courrier (1927) to the effect that in all species examined by him *some* involution of the sex accessories has already begun in the fall, but in a graded fashion in different species; it is extreme in *Miniopterus*, intermediate in *Myotis*, and minimal in *Pipistrellus* and *Eptesicus*. Also suggestive is the sudden involution which in all species overtakes the sex accessories immediately after hibernation terminates in the spring. On the other hand, the hypothesis is apparently contraindicated by the results of some experiments of Courrier and Pearson *et al.*, both of whom were able to demonstrate, in *Eptesicus* and *Corynorhinus* respectively, that the sex accessories involute more rapidly in males removed from hibernation and bilaterally castrated, than in non-castrated controls. Also more rapid natural involution during the fall of the sex accessories of *Miniopterus* than those of other species would militate against the hypothesis, if *Miniopterus* enters hibernation when the others do.

However, none of the above experiments preclude the further possibility that small but effective quantities of androgen produced during the period of breeding activity preceding hibernation might persist in the testes or elsewhere, e.g. in brown fat, unmetabolized in the torpid animal, and be available to retard involution of the sex accessories in non-castrated activated males. It is significant in this regard that the hibernating state alone will prevent involution of the sex accessories in the face of abrupt androgen withdrawal, for Courrier (1927) and Wimsatt (unpublished observations) have observed that if hibernating specimens of *Eptesicus* are bilaterally castrated, and returned immediately to hibernation,

no detectable involution of the glands occurs, even over a period of several months. The possibility of a retarded utilization of "residual" hormone under the influence of hibernation would seem not to be essentially different in concept from this demonstrated retarding effect of hibernation on the involution of the male glands, and furthermore it receives indirect support from other evidence to be presented shortly. Obviously, many aspects of the endocrine cycle of the testes in hibernating bats require further experimental study.

A second, potentially significant, unsolved problem in the male concerns the physiological mechanisms (endocrine and neural) which condition the *apparent* asynchrony between the spermatogenic and endocrine cycles of the testis. The word "apparent" is emphasized because the asynchrony will be real only if it turns out that the testis continues to elaborate androgen during the hibernation period, when the seminiferous tubules are quiescent. If this proves to be the case, it would appear likely that the primary cause may reside in the asynchronous release of hypophyseal gonadotrophins (FSH and LH) known in other mammals to be separately responsible for stimulation of the two gonadal functions. An indication that separate release of gonadotrophins can occur is provided by an observation of Courrier (1927). He describes a "eunuchoid" *Pipistrellus*, collected in September, in which spermatogenesis had been normal (epididymal sperm) though now terminated, but in which the sex accessories, which should have been fully hypertrophied, were totally undeveloped; the interstitial tissue was likewise fully involuted.

It is conceivable that an asynchronous production of gonadotrophins might be reflected by alterations in the cytology of the hypophysis. To date only a single definitive study of pituitary cytology in the male bat has been carried out, by Siegel (1955), who worked on *Myotis lucifugus* in my laboratory. He was the first to demonstrate that in the bat, as in other common laboratory mammals, the classical "basophiles" are divisible into two functionally-specialized groups, "thyrotrophs" (producing TSH) and "gonadotrophs" (producing gonadotrophins). He was unable to subdivide the "gonadotrophs" further, however, and his study produced no evidence, either for, or against, the concept of an asynchronous production of different gonadotrophic hormones. The problem is one which requires further study, particularly in conjunction with the working out of the true endocrine status of the testis in the hibernating male. We shall turn next to conditions in the female.

The female bat. Among the many problems of reproductive physiology in female bats, two seem to me to be most provocative, namely, the mechanisms of ovulatory delay, and of sperm survival in the female tract. I shall be able to consider here only the first. Several questions might conceivably be asked concerning the characteristic delay of ovulation: What endocrine mechanisms condition follicular growth and ovulation in bats? Are these subject to neural influences, and if so, of what kind? Does hibernation *per se* have any direct effect on the operation of these factors? And, finally, what is the mechanism for reactivation of the interrupted cycle at the end of hibernation?

A priori it appears unlikely that the immediate factors controlling reproductive phenomena in bats differ in fundamental quality from those operative in other mammals, for the reproductive organs of bats respond in the same ways to gonadotrophic and sex hormones. It seems most reasonable to suppose that the prolongation of the proestrous phase through hibernation results from the interplay of at least three factors: the depressing effects of hibernation itself on cellular metabolism and reactivity; a probable dissociation in time of hypophyseal gonadotrophic functions which in other mammals are more closely synchronized; and a neural mechanism which may regulate hypophyseal (gonadotrophic) function and itself be triggered by environmental and/or internal stimuli. The timing of the cycle in the female and experimental results to date indicate the possible involvement of all of these.

As mentioned earlier, the ovary of the hibernating bat typically contains throughout the period of dormancy a viable vesicular follicle, or follicles, which are destined to experience preovulatory growth and to rupture shortly after hibernation ends in the spring; no other large follicles are present. The prolonged survival of these follicles is unique, and it is perhaps not remarkable that they should demonstrate a chemical specialization not observed in the follicles of other mammals. It consists in a pronounced vacuolation of the discus cells surrounding the ovum (Plate, fig. 1) which is brought about by the deposition within them of enormous quantities of glycogen (Plate, fig. 2). Pearson *et al.* (1952) have adduced evidence to suggest that in *Corynorhinus* this characteristic vacuolation of the discus cells is dependent upon copulation, but Wimsatt and Kallen (1957) were able to show that in *Myotis lucifugus*, at least, it occurs in the absence of a copulatory stimulus. They postulated that it is an adaptive response to hibernation itself, the glycogen representing a readily available source of energy providing for the

survival of the follicle under conditions of a drastically reduced metabolism in the hibernating animal; experiments are currently in progress to test the validity of this concept.

But our immediate problem is to determine the reasons why this follicle does not complete its maturation and go to rupture before spring. Experimental results indicate that at least two interrelated factors may be involved, and perhaps more. The first is an inability of the follicle to respond to hormone stimulation while the bat is in a torpid condition, and the second is the probable absence during hibernation of sufficient gonadotrophic hormone (LH) to precipitate ovulation. These conclusions are based on the results of personal experiments partially summarized in Table I, which records the effects of various hormone injections on induction of ovulation in *Myotis* under different conditions and at different times during the winter. First it can be seen that ovulation was elicited by all pituitary hormones used except ACTH and oxytocin, but it was effected most easily and consistently by the FSH preparation. One may perhaps suspect that the TSH and Growth hormone preparations contained some gonadotrophin contaminant. Secondly, it should be noted that ovulation was induced only in animals which were maintained at room temperature during the experiments. In the group of FSH-injected animals maintained in a torpid condition throughout the experiment not a single ovulation was induced, which demonstrates that the ovary is unable to respond to circulating hormone in the torpid animal. Significantly, removal of injected torpid animals to room temperature was promptly followed by ovulation induced by the residual hormone previously administered. Thirdly, thyroxine (Tx) and cortisone were ineffective in eliciting ovulation in animals maintained at room temperature, although thyroxine did induce some follicular enlargement and glycogen discharge from the discus cells, but without maturation changes in the ovum.

On the basis of these findings the following reasoning seems plausible: Since ovulation can be quickly precipitated by pituitary gonadotrophins at any time during the hibernation period (provided a surviving follicle is present), and hormones which merely influence the general level of metabolism are ineffective, there is at present no reason to assume that preovulatory growth and ovulation are not normally under direct hypophyseal control. Furthermore, it is well known that hibernating bats brought to room temperature during the early months of hibernation will not ovulate spontaneously (Guthrie, 1933; Wimsatt, 1944b).

TABLE I

Effects of Hormone Injections on the Induction of Ovulation in the Bat *Myotis lucifugus lucifugus*.*

		EXPERIMENTS: Injected #	Injected Horm.	Controls #	Controls Horm.	DOSE p/d	DATE INJECTED	SACRIFICE	OVULATION RESPONSE: Injected	Controls	REMARKS
HYPOPHYSEAL HORMONES	ACTIVE (25°C)	3	OXYTOCIN	4	—	0.125 I.U.	2/5	2/6	−(3)	−(4)	
		3	ACTH	3	—	2, 1, 2 I.U.	1/27 1/28 1/29	1/31	−(3)	−(3)	
		2	PROLACT	4 ↓	—	5 I.U.	1/14	1/18	+(1), −(1)[1]	−(4)	[1] no lge. foll. in ovaries
		2			—	5 I.U.	1/14 1/15	1/18	+(1), −(1)[2]	sacrif. 1/18	[2] lut. foll. in ovary
		2			—	5 I.U.	1/14 1/15 1/16	1/18	+(1), −(1)[3]	↓	[3] norm. foll. in ovary
		3	GROW HOR.	3	—	1 mg.	1/27 1/28 1/29	1/31	+(3)	−(3)	
		3	FSH & Tx	3	—	.5 A.U. & 1.25 mg	1/27 1/28 1/29	1/31	+(3)	−(3)	
		4	TSH	4	saline	4.5	4/11 4/12	4/14	+(4)	−(3), +(1)[4]	[4] spontan. (time of yr.)
		4		4	saline	J.S.U.	4/11 4/12 4/15	4/15	+(3), −(1)[5]	+(2), −(2)[6]	[5] preovulatory gr. [6] no lge. foll. (young)
	TORPID (2°C)	4	TSH	4	saline	4.5	4/4 4/12 4/14	4/14	−(4)	−(4)	
		4		4	saline	J.S.U.	4/11 4/12 4/15	4/15	−(4)	−(4)	
	ACTIVE (25°C)	4	FSH	see "Remarks"		.75 A.U.	3/4		+(4)	−(12)[7]	[7] controls = all torpid bats
		4				.75 A.U.	3/7 ret to cold 3 da then rm. temp.		+(4)	↓	
	TORPID (2°C)	4	FSH	see "Remarks"		.75 A.U.	3/4		−(4)	+(8)[8]	[8] controls = all active bats
		4				.75 A.U.	3/7		−(4)	↓	
		4				.75 A.U.	3/4		−(4)		
NON-HYPOPH HORMONES	ACTIVE (25°C)	4	Tx	4	—	1.25 mg	1/14 1/15 1/16	1/18	−(4)	−(4)	
		3		2	—	5.0 mg	2/5 − 2/11 incl.	2/13	−(3)	−(2)	
		3	INSUL	4	—	.25 IU.	2/5	2/6	−(3)	−(4)	
		3	ADREN	4	—	165 IU.	2/5	2/6	−(3)	−(4)	
		7	CORT.	see "Remarks"		1 mg.	3/23 3/24	3/25	+(2), [9]−(5)	− 8	[9] controls = all torpid bats spontan. (time of yr.)
	TORPID (2°C)	8	CORT.	see "Remarks"		1 mg.	3/23 3/24	3/25	−(8)	+(2), [9]−(5)	controls = all active bats

Hypophyseal Hormones courtesy Armour Laboratories, Chicago

*Based on unpublished data

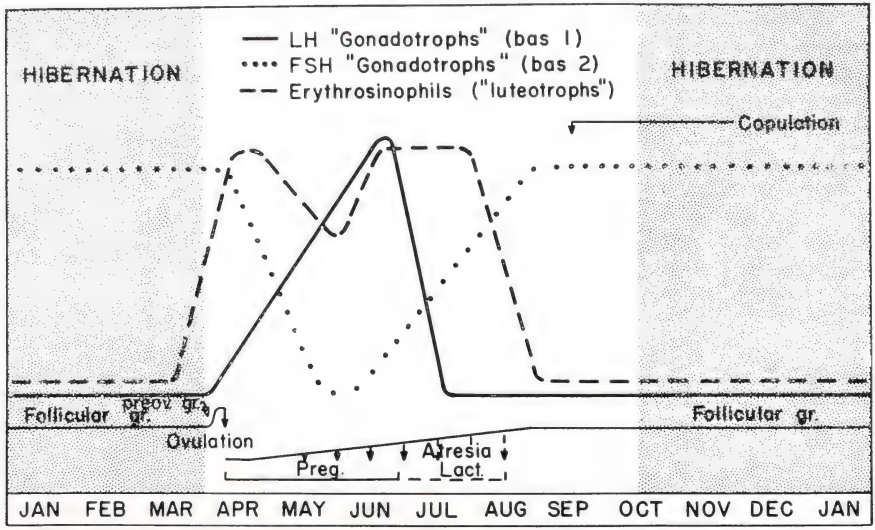
which suggests that ordinarily no "residual" gonadotrophin is present, and that an increased metabolism, *per se*, does not stimulate the hypophysis to produce any. In view of the effectiveness of injected hypophyseal extracts in promoting ovulation at this time, it seems reasonable to conclude that insufficient gonadotrophin is one condition of ovulatory delay in hibernating bats. It is also known (*loc. cit.*) that the natural refractory status (to ovulation) of the fall and early winter periods is gradually lost during the later months of the hibernating period, for artificial arousal in the later months often results in spontaneous ovulation, and this occurs with increasing frequency as the end of the hibernation period is approached. This phenomenon might be explained by assuming that later in the hibernation period ovulating hormone slowly produced by the pituitary of the torpid animal has accumulated to the point where it exceeds the follicular threshold of response and can therefore precipitate ovulation in the awakened animal. A possible objection to this assumption is the question as to whether the pituitary is even capable of carrying out secretory functions at the low body temperatures of the torpid bat. It seems more reasonable to consider that the ovulatory function of the hypophysis of the bat, as in many other mammals, may be dependent upon a stimulus from the hypothalamus which in turn is activated reflexly by intrinsic or environmental stimuli. The very periodicity of reproduction in bats suggests, *a priori*, the existence of such a neural mechanism, sensitive perhaps to as yet unknown environmental stimuli.

Two further questions remain in respect to the mechanism of delayed ovulation which I should like to discuss briefly. One concerns the probable identity of the specific gonadotrophic hormone which is presumably lacking during hibernation, and the other concerns further aspects of the neural mechanism through which its release may ultimately be effected. While ovulation was readily elicited with a commercial FSH preparation, it does not follow that FSH is the ovulating hormone in bats any more than the similar results obtained with Prolactin, TSH or Growth hormone indicate that any of these are the physiological ovulator. The common denominator in all of these pituitary preparations could well be contaminating amounts of luteinizing hormone (LH), the agent which is known in other mammals to precipitate ovulatory responses in conjunction with FSH. The very presence of the large follicle of hibernation presupposes that FSH has already exerted its effects before the bat enters

hibernation, and in all probability some "residual" FSH is still present in the system of the hibernating female. Herlant (1956) has recently produced some indirect evidence which suggests that LH may be the specific ovulating hormone in the bat, and which lends strong support to the notion that the ovulating hormone is normally produced only at the end of hibernation, or in bats prematurely aroused in the spring. Herlant's work is a magnificent study based on a correlation between marked seasonal changes in pituitary cytology and the annual cycle of reproduction in the female bat *Myotis myotis*. His results are summarized in Figure 2. He was able to demonstrate that the anterior hypophysis contains five distinct types of chromophilic cells; transitional types were not discerned. Three of these, basophils 1, 2 and 3 respectively, are distinct subtypes of the classical "basophiles", and two, acidophils 1 and 2, belong to the category of classical "acidophiles." All three of the basophils are PAS-positive, and "basophil 3" is, in addition, positive to aldehyde-fuchsin. On the basis of cyclic changes in the number and staining intensities of these cells correlated with the reproductive cycle on the one hand, and the annual cycle of activity on the other, Herlant was able to deduce the probable functional specificity of all five chromophilic cells. The aldehyde-fuchsin cell (basophil 3) secretes TSH as in other mammals, and acidophil 2 probably secretes ACTH and Somatotrophin. The remaining three cells are "gonadotrophs", basophil 1 secreting LH, basophil 2 FSH, and acidophil 1, which is erythrosinophilic, luteotrophin (prolactin). In the figure the cyclic changes in these cells are shown to be correlated with the various events of the reproductive cycle known in other mammals to be conditioned by these gonadotrophic hormones. Thus, basophil 1, which is presumed to secrete LH, hypertrophies at the time of ovulation, attains maximum development during gestation, and undergoes massive involution following parturition, coinciding with the degeneration of the corpus luteum. Basophil 2 is presumed to secrete FSH. It hypertrophies at the time of the autumn rut, remains well developed through hibernation, but involutes during early pregnancy. The functional specificity of this cell is further attested by the solubility of its granules in trichloroacetic acid (cf. Ladman and Barrnett, 1955). The erythrosinophil, which is presumed to secrete luteotrophin, shows two phases of activity; the first coincides with ovulation, and is followed by a brief involutionary phase, while the second which begins in late pregnancy, is contemporaneous with lactation. In females failing to lactate, this second

FIGURE 2

GONADOTROPHIC CELLS OF HYPOPHYSIS ♂ *MYOTIS* *MYOTIS* ♀



Modified from Herlant

peak of erythrosinophils is not observed. Herlant's beautiful study should be confirmed in other species of hibernating bats, and if such confirmation is forthcoming it will provide strong presumptive evidence that the long sought endocrine basis of delayed ovulation in bats has been found.

It is common knowledge that in many vertebrates seasonal reproductive periodicity is conditioned by external environmental stimuli, the responses being mediated through a neural mechanism involving the activation of a "sex center," presumably in the hypothalamus, by incoming sensory impulses. It has also been demonstrated that specific reproductive events may be dependent upon appropriate "psychic" stimuli, as for example the dependence of ovulation on a coital stimulus in the so-called "induced ovulators." The basic mechanism is presumably the same in both instances, activation of the gonadotrophic function of the hypophysis either by neural, or neuro-humoral stimuli from the hypothalamus. Recently, evidence has been presented (Sawyer, *et al.*, 1949; Everett, 1952) which indicates that even in spontaneously ovulating mammals, such as the rat, the release of ovulating hormone (LH) may be elicited by a neuro-chemical mediator arising in the hypothalamus, and that estrogen is an effective stimulus for its release. The mechanism in the rat appears to differ from that in the rabbit only in its spontaneity. Furthermore, estrogen-induced ovulation in pregnant rats is blocked by anti-adrenergic and anti-cholinergic drugs (pentobarbital and atropine respectively) if these are administered soon after the estrogen injection.

At present nothing definite is known concerning possible neural involvement in the reproductive processes of hibernating bats, but the characteristic reproductive periodicity and the asynchronous release of pituitary gonadotrophins implied in Herlant's (1956) work suggest strongly that neural mechanisms may in fact be involved. Taking his cue from the work of Sawyer *et al.* (1949) and Everett (1952), Herlant (1954) recently attempted to determine whether raising the estrogen levels in hibernating bats (*Myotis myotis*), which are in a state of "sub-estrum" during the winter sleep (Guthrie and Jeffers, 1938; Wimsatt, 1944b), would stimulate the ovulation reflex. Eight adult females were procured in February and maintained at 15°C in the laboratory. Each bat received subcutaneously 0.1 mg estradiol proprionate at 48 hour intervals, and were autopsied 2 to 10 days after the first injection. Seven of the eight injected animals ovulated, as did one of the uninjected controls, although Herlant did not indicate

how many control animals were used. The results are highly suggestive, but unfortunately were carried out too late in the year to preclude the possibility that spontaneous ovulation had occurred in the activated animals. Should similar experiments carried out earlier in hibernation, when spontaneous ovulation does not occur, prove successful, and especially if the response is blocked by adrenergic drugs, strong presumptive evidence would be provided that LH release in the bat, as in the rat and rabbit, is mediated by a neuro-chemical mechanism. If this can be established, then the way is open for an experimental analysis of the environmental and/or intrinsic factors which activate the hypothalamic response, and the eventual elucidation of the complete physiology of delayed ovulation in hibernating bats.

Summary

The reproductive cycles of hibernating bats display many peculiarities which undoubtedly have arisen in consequence of the evolution of the hibernating habit and the superimposition of the period of hibernation on the season of reproduction, for corresponding peculiarities are not observed in tropical, non-hibernating bats. While the annual sequence of reproductive events has been well worked out for many species, an integrated picture of the underlying endocrine and, possibly, neural mechanisms involved has not yet been achieved in any bat, nor have the immediate effects of hibernation *per se* on these mechanisms been adequately studied. This paper presents a review of the reproductive peculiarities of hibernating bats, and focuses attention on what, in the author's opinion, are some of the fundamental problems needing further experimental analysis. These include: in the male, determination of the true endocrine status of the testis during hibernation, and related to this, an investigation of the *apparent* asynchronous production of pituitary gonadotrophins regulating the two aspects of gonadal function (production of spermatozoa and androgen respectively); and in the female, elucidation of the physiological mechanisms underlying the central peculiarity, the delay of ovulation through hibernation, and the specific influences of hibernation and external stimuli on the operation of these mechanisms. Recent experiments by the author and others which indicate their probable nature are discussed.

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DISCUSSION FOLLOWING WIMSATT'S PAPER

HOCK pointed out that it is not quite true to believe that ground squirrels show no reproductive or embryological changes during hibernation, and that breeding takes place only in the spring. He noted that the earliest appearing hoary marmots in the spring have been found to have two 2.5 cm embryos in place, which means that breeding must have occurred the previous fall.

WIMSATT replied that he did not know of this, but realized that some early spermatogenic changes occur in the woodchuck and hedgehog prior to hibernation but that these are still essentially spring-breeding species. He emphasized that the main fact to remember is that except in bats hibernation is a period of sexual quiescence.

HOCK indicated that spermatogenesis has proceeded to the secondary spermatocyte stage before animals enter hibernation.

MENAKER asked if bat sperm had been stored *in vitro* at temperatures close to those encountered in hibernation. WIMSATT knew of no such work and remarked that, if required, collecting sperm from a female reproductive tract would be a considerable task. LYMAN asked what the distribution of sperm was in the female bat reproductive tract. WIMSATT replied that most of the sperm were located in the fundi of the uterine glands and in the uterine lumen; usually they are oriented perpendicularly with their heads against the epithelium.

BRATTSTROM stated that Wade Fox (unpublished observation) has shown that female garter snakes (*Thamnophis*) can retain and nurture sperm in special sacs for at least six months.

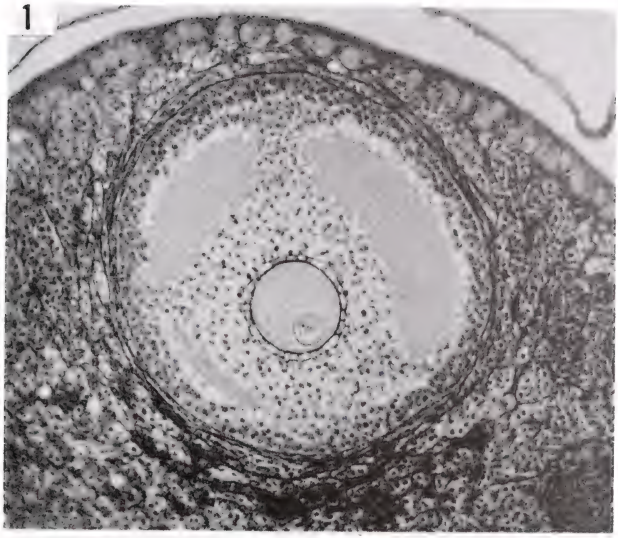
SCHÖNBAUM pointed out that JOHANSSON had mentioned the presence of a large quantity of androgen in brown fat, but in rodents. Were androgens in high titer in bat brown fat? WIMSATT replied that it was possible, but again he knew of no evidence for this.

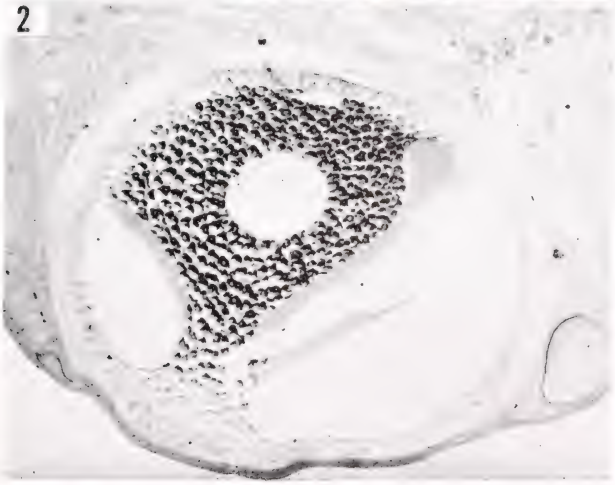
FOLK pointed out that he and Grindeland had information which indicated that the estrous cycle of the hamster continues into hibernation. He asked WIMSATT to comment on this. WIMSATT replied that sex organs proved remarkably unresponsive in the torpid animal in experiments using gonadotrophic hormones, and that if torpid tissues are unresponsive to hormones he could not see how hormones could initiate arousal or "precipitate anything" in the torpid animal. He stated that his experiments took place during a short term and do not rule out the possibility of hormonal effects over a long period in the hibernating animal.

PLATE

Fig. 1. Surviving follicle of hibernation in the ovary of the bat *Myotis lucifugus lucifugus* showing the characteristic vacuolation of the cells of the discus proligerus. The follicle maintains this appearance throughout the hibernation period, and only experiences preovulatory growth and rupture after the bat emerges from hibernation in the spring (Hematox. & eosin).

Fig. 2. Surviving follicle of hibernation in the bat *Eptesicus fuscus fuscus* illustrating the rich deposits of glycogen responsible for the vacuolation of the discus cells (Bauer-Feulgen stain).





Plate

XIV

STRESS AND NEUROSECRETION IN THE HIBERNATING HEDGEHOG

By Paavo Suomalainen
Department of Physiologic Zoology
University of Helsinki
Helsinki, Finland

In recent years, Selye's theories (1950) concerning the adaptation syndrome and the hypophyseo-adrenal system have aroused considerable controversy, but have also shed new light on many problems.

If an individual is continuously exposed to stress, the resulting adaptation syndrome evolves, according to Selye (1950), in three stages: (1) an initial alarm reaction, which is defined as the sum of all the non-specific systematic phenomena elicited by sudden exposure to stimuli to which the organism is not adapted; (2) the stage of resistance, adaptation proper, when the body's compensatory reactions to stress develop; and (3) a phase of exhaustion which occurs if exposure to stress is excessive.

Agents causing merely local damage, which requires no general adaptive adjustment, are relatively mild alarming stimuli, while those which evoke intensive adaptation responses produce severe alarm reaction symptoms. Examples of the latter are cold and fasting, both characteristic of hibernation.

The adaptation syndrome is characterized by a number of morphologic and functional changes. A convenient indicator of stress is the blood picture. In the south of Finland, hedgehogs are in their normal summer condition from the middle of June to the middle or end of August. On account of the pregnancy of the females in late May and in June we have investigated only males at this season. Between these summer hedgehogs and hibernating animals there are remarkable differences (Table 1). During hibernation we find all the blood changes typical of stress — leucopenia, neutrophilia, eosinopenia and lymphopenia. But the animals investigated in the autumn, before the onset of hibernation, and in the spring when they have wakened up are also interesting. In the autumn there is already a clear neutrophilia, eosinopenia and lymphopenia. In the south of Finland, hedgehogs emerge from hibernation in the beginning

of May. After this the figure for neutrophils is almost the same as in the autumn. The blood count shows a clear neutrophilia and lymphopenia without eosinopenia. Hedgehogs brought into artificial hypothermia in a refrigerator in July resemble those in natural hibernation. The marked "shift to the left" during hibernation indicates that the bone marrow is active even in hibernating animals.

TABLE I

Leucocyte Picture of the Hedgehog

	WBC per cu. mm.	Neutrophils % Band form	Neutrophils % Segmented	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %
In spring	18,700	2.6	47.4	6.1	1.6	38.5	3.8
In summer	18,100	1.8	35.0	5.1	2.0	55.1	1.0
In autumn	17,700	5.7	42.3	2.1	0.9	48.3	0.7
In hibernation	4,100	47.5	28.3	1.2	0.8	20.9	1.3

To judge from the blood count, the adaptation syndrome is already very pronounced in the autumn, even before the onset of hibernation (cf. the low figure for eosinophils). In hibernating animals the adaptation syndrome is at its most intense. In the spring when the animals emerge from hibernation, the stress picture is less.

In response to the various stress stimuli the body shows a common syndrome, which includes discharge of adrenal hormones, hypertrophy of the adrenal cortex, involution of the lymphatic system and disturbances of the gastrointestinal tract, kidney and other organs (Selye, 1950).

Table II shows the variations in the weight of the adrenal glands relative to the body weight at different seasons of the year. The adrenal glands are distinctly enlarged during hibernation. We have also estimated microscopically, from sections made as near the median line as possible, the ratio of the area of the cortex to the area of the medulla and to the whole adrenal gland. The figures in Table II show that it is the cortex which is especially enlarged during hibernation. The clearest change is in the zona reticularis.

Autumn and spring are obviously times of stress for animals and also for man. The transition from the summer condition to

the winter condition and vice versa is not at all easy from the physiological point of view. And these seasons are still more difficult for animals that hibernate; their heat balance is then radically changed. In response to the stress stimuli the adrenal cortex has an important part to play. In the histophysiologic investigation of the adaptation syndrome, attention was chiefly devoted to the thickness of the adrenal cortex, and to the number and position of the lipid granules in the cortex.

TABLE II

Relative Size of the Adrenal Gland and of the Adrenal Cortex in the Hedgehog

	Relative adrenal weight (mgm/100 gm body-weight)	Area of cortex/ area of the whole adrenal gland	Area of cortex/ area of medulla
In spring	53		
In summer	50		
In autumn	53	0.86	7.0
In hibernation	62	0.93	14.2
In early spring	52		

In the autumn the histologic picture of the cortex is already changed (Suomalainen, 1954). The cortex is certainly not yet enlarged and its zones can be clearly distinguished from each other. But the lipid granules are situated in the zona reticularis (in summer chiefly in the zona fascicularis), and are not found in other zones. A decrease in the sudanophilic substance is a sign of increased activity and a slight alarm reaction.

In adrenals investigated a couple of weeks before the onset of hibernation, an exceptionally large number of degenerating cells were found in the cortex (Suomalainen, 1954). The amount of lipids was increased in all zones of the cortex, but especially in the zona glomerulosa. The cortex had passed into the resistance stage of the adaptation syndrome.

During hibernation, the resistance stage seemed to continue (Suomalainen, 1954). The cortex was clearly swollen and there were no sharp boundaries between the zones. The zona reticularis was enlarged and the cells of the zona fascicularis slightly swollen. The degeneration of the cells was evidently very marked. The lipids were concentrated in the outer parts of the

cortex and elsewhere diminished, but they were still present in large amounts. In deep hibernation, in the cold of midwinter when the body temperature had fallen as low as +2 to 4°C, the cortex, with the exception of the zona glomerulosa, was very disorganized. The amount of lipids had decreased everywhere, and the lipids seemed to have broken up into very fine droplets. The activity of the gland was obviously increased.

In the hedgehog awakened from deep hibernation in winter, the zona reticularis was very cavernous, and even the zona fasciculatis was spongy and contained cells that were in the process of degeneration (Suomalainen, 1954). The lipid granules had almost entirely disappeared, but sometimes they were present in the zona glomerulosa as very fine droplets. The histologic picture corresponded either to a strong alarm reaction or to the exhaustion stage of the adaptation syndrome.

In addition to the decrease in the sudanophilic substance of the adrenal cortex, a simultaneous decrease in the sudanophilic substance of the hibernating gland of the hedgehog can be demonstrated (Suomalainen, 1954). Investigations made in my department have shown that the sudanophilic substance decreases in the hibernating gland, especially when the animals emerge from hibernation. At the same time the granules are greatly reduced in size. This, too, shows that emergence from hibernation is a severe physiologic stress.

TABLE III

Cholesterol Content (mgm-%) of the Adrenals, Hibernating Gland and Serum of the Hedgehog

	Total cholesterol			Cholesterol esters		
	Adrenals	Hibernating gland	Serum	Adrenals	Hibernating gland	Serum
In spring	772	173	193	478	29	137
In summer	1103	218	236	698	50	155
In autumn	1580	223	274	1042	36	138
In early winter	773	198	199	318	32	196
In midwinter	895	209	233	503	23	170

It is interesting to compare cholesterol determinations made from the adrenals and hibernating gland with the histophysiologic investigations. According to Selye (1950) and others, the cholesterol content of the adrenal cortex is reduced during the

alarm reaction and the exhaustion stage of the adaptation syndrome, and is normal or increased during the stage of resistance. Table III shows the means of our determinations. In the autumn the histologic picture of the adrenals revealed a slight alarm reaction. At the same time their cholesterol content was slightly reduced. In midwinter the hibernating hedgehogs, according to the histologic investigations, were in the resistance stage. The cholesterol content was then slightly increased. The cholesterol content of the hibernating gland parallels the changes in the cholesterol content of the adrenal glands.

TABLE IV

K^+ Content of the Blood and Na^+, Mg^{++} and Ca^{++} Content of the Serum in the Hedgehog (mgm/100 ml)

	K^+	Na^+	Mg^{++}	Ca^{++}
In spring	160	393	4.5	10.1
In summer	161	389	3.4	10.3
In autumn	127	393	4.0	10.0
In hibernation	104	418	5.8	10.4

In an intact animal the administration of mineralocorticoids influences the level of potassium and sodium in the plasma; the plasma is depleted of potassium, and the sodium concentration increases. As is evident from Table IV, the K^+ content of hedgehog blood is already reduced in autumn before hibernation and reaches a minimum during hibernation (Suomalainen, 1956). The Na^+ content of the serum, on the other hand, is slightly raised. Hibernation does not appear to have any effect on the serum Ca^{++} content. A feature typical of hibernation in the hedgehog, and also of hypothermia in many poikilothermic animals, is an increase in the Mg^{++} content of the blood.

TABLE V

The Relative Size of the Lymph Nodules (or Follicles) in the Cortex of the Lymph Node in the Hedgehog

	In the neck	In the groin
In autumn	179	205
In early winter	99	144

The general adaptation syndrome with adrenocortical enlargement is usually accompanied by an involution of the lymphatic organs (Selye, 1950). Table V shows the means of the relative size of the lymph nodules in the cortex of the lymph nodes in the neck and in the groin of active hedgehogs and of hibernating ones.

The control of the adaptation syndrome is complicated. It is obvious that the hypophysis plays a central part through secretion of the adrenocorticotropic hormone (ACTH) following stress. Again, many experiments of recent years (Scharrer, 1956; Bargmann *et al.*, 1958) make it appear likely that nerve fibers from the anterior part of the hypothalamus terminating around the multitude of capillary loops in the median eminence of the tuber cinereum liberate some chemical mediator into the blood by which it is conveyed to the adenohypophysis to regulate its secretory activity. Under conditions of stress the liberation of ACTH appears to be excited by nervous influences acting on the hypothalamus both directly and indirectly through neural pathways from the cortex and the anterior nuclei of the thalamus. Stress may also effect ACTH secretion through variations of the blood supply to the hypophysis. It is also likely that adrenaline released from the adrenal medulla in stressful states may have a subsidiary effect in exciting secretion of ACTH. But there is some evidence that insulin, by facilitating the passage of glucose into the cell, plays a part in the reaction to stress.

The investigations of my department (Suomalainen and Uuspää, 1958) make it evident that the adrenaline content of the adrenal glands of the hedgehog usually increases just at the time when the cortex, too, is activated on account of physiologic stress (Fig. 1). This is especially the case during periods of intense cold in midwinter. But even in autumn, before the onset of hibernation, the ratio of adrenaline to noradrenaline has already increased. From the investigations made in my department it seems likely that the production of insulin, too, is increased in hibernating hedgehogs (Suomalainen, 1956).

The nature of the humoral mediator (or mediators) liberated in the median eminence of the tuber cinereum and involved in the release of ACTH is at present unknown. It is probable that it is a polypeptide component of neurosecretory material, liberated by the nerve endings in the median eminence and infundibular stem (Suomalainen and Uuspää, 1958).

Neurosecretory cells are nerve cells which show cytologic evidence of secretory activity. They elaborate microscopically visible granules and droplets, which, in most cases, pass along the axons toward the nerve terminals. In vertebrates, the nerve terminals in which the neurosecretory material is stored make up a great part of the posterior lobe of the hypophysis (Scharrer, 1956; Bargmann *et al.*, 1958).

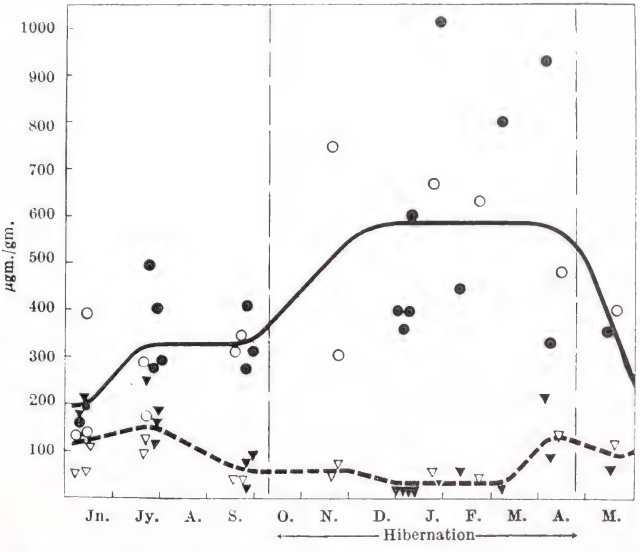


Fig. 1. Adrenaline and noradrenaline content of the adrenal glands of the hedgehog in different seasons. ——, Adrenaline, ● (♂), ○ (♀); – – –, noradrenaline, ▼ (♂), ▽ (♀). From Suomalainen and Uuspää (1958).

The neurosecretory substance stains characteristically with chrome-alum-hematoxylin-phloxin and aldehyde-fuchsin. It is a complex protein to which active polypeptides, such as vasopressin and oxytocin, may be attached (Scharrer, 1956; Bargmann *et al.*, 1958).

Neurosecretory cells occur in various parts of the nervous system. In mammals the neurosecretory cells of the hypothalamus form two conspicuous groups, the nucleus supraopticus and

the nucleus paraventricularis. In all vertebrates studied so far, the axons of these two nuclei form a conspicuous fiber tract, the tractus hypothalamo-hypophyseus, which descends toward the hypophysis. In mammals, some of the fibers terminate in the pars intermedia. The great majority of the axons arising from neurosecretory cells in the hypothalamus end around blood vessels in the neurohypophysis. The neurosecretory material contains the hormones, which were formerly thought to be produced by the posterior pituitary (Scharrer, 1956; Bargmann *et al.*, 1958).

Because objective quantitative estimation of the neurosecretory substance in microscopic slides is almost impossible, we have used the volume of the cell nucleus as the index of its activity (Suomalainen and Nyholm, 1956). Using the camera lucida, 100 cell nuclei, magnified 1500 times, were drawn from the nucleus supraopticus of each animal. The sizes of the nuclei were measured with a planimeter. The mean values are given in Table VI.

The Table shows that the cell nuclei are at their smallest in June. In July-August and in the pre-lethargic period of September-October the nuclei steadily increase in size. In hibernating animals they are always bigger than in active ones. They are largest in March and then grow smaller during the post-lethargic period in April when the animals are still hibernating.

The picture of the nucleus supraopticus-hypophyseal system parallels these conclusions. In summer hedgehogs there is hardly any secretory substance in most of the cells. The axons of the hypothalamo-hypophyseal tract stain poorly. At the distal end of the tract are the Herring bodies, that is, axons with bulbous swellings, containing considerable amounts of secretory substance. There is often a moderate quantity of this substance in the neurohypophysis, also. The distal end of the latter, however, frequently contains no secretory substance. We may conclude that neurosecretion is rather slight in summer.

In the pre-lethargic period in autumn the nuclei of the nucleus cells have already increased in size. A large amount of the secretory substance may even be present, in the form of large droplets and coarse granules (Plate, fig. 1). There are also cells containing less of the secretion, in finer granules. In the intercellular substance there are often copious accumulations of secretory granules. The axons of the tract may be quite thick and intensely stained. Furthermore, there are dark Herring

bodies, both large and small (Plate, fig. 2). Conclusion: Neurosecretion is more active, but the secretory substance is still stored in the hypophysis.

TABLE VI

The Relative Size of the Cell Nuclei in the Nucleus Supraopticus at Different Seasons

Awake			*Hibernating*		
In June	2.97		In November		
	2.60		December	3.80	
	3.50	3.02		3.85	
				4.26	
In July	3.46			4.42	4.08
	3.52				
	2.93	3.30	In January	4.80	
				5.34	
In August	3.58			4.28	4.81
	3.63				
	3.68	3.63	In February	4.62	
				5.35	
In September—				4.53	4.83
October	3.44				
	3.82		In March	5.05	
	3.63			4.75	
	4.35			5.40	5.07
	3.73	3.79			
			In April	4.61	
				4.30	
				4.30	4.40
			Awake		
			In May	3.41	
				3.50	
				2.89	3.27

From Suomalainen and Nyholm (1956).

In the hibernation period, the nuclei of the nucleus cells and the cells themselves, too, are large (Plate, fig. 3). The neurosecretory substance is present as very fine granules, if it is visible in the cells. The axons of the tract are thin but stain well. They are most evident at the distal end of the tract. The capillaries at the proximal end of the neurohypophysis are greatly enlarged. Conclusion: Neurosecretion is intense during hibernation. The secretory substance is no longer stored in the neurohypophysis except to some extent in midwinter.

In general, we can say that there is a clear correlation between neurosecretion and stress in hedgehogs all the year round. Among the recent surveys which have emphasized that the neurosecretory substance contains the hormone stimulating the adenohypophysis, I would especially mention the contribution of Martini (1958) and Saffran *et al.* (1958) to the second international symposium on neurosecretion at Lund in 1957, and the investigations of Guillemin (such as 1956).

From investigations performed during the present decade it is obvious that secretory activity also occurs in the subcommissural organ (Scharrer, 1956; Bargmann *et al.*, 1958). This organ constitutes a specialized area of the ependyma located beneath the posterior commissure of the midbrain in the roof of the third ventricle, in the region where the ventricle suddenly narrows to become the cerebral aqueduct. Such an organ has been reported from all the vertebrates investigated in this respect, with the exception of a few mammals. In some cases, among the mammals, subcommissural cell groups have withdrawn from the ventricular position and have formed a glandular island beneath the ventricular epithelium. They may also abandon the ancient mode of secretory release into the ventricle and probably give off their material into blood vessels. The subcommissural organ of the hedgehog is apparently of this type. In Figure 4 (Plate) you see a cross-section through this great group of cells. We have demonstrated that they contain a substance which stains like the neurosecretory substance (Plate, fig. 5), and which even forms Herring bodies (Plate, fig. 6). We have not yet investigated whether there is any seasonal rhythm in this secretion or in the changes in the size of the nuclei which secrete it. But even the discovery of a new center of secretion in the brain is interesting.

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DISCUSSION FOLLOWING SUOMALAINEN'S PAPER

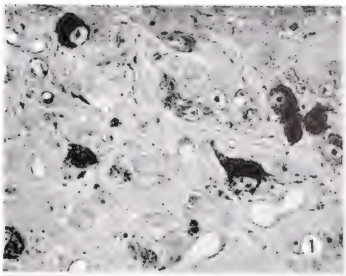
BULLARD inquired as to relative versus absolute increase in size of glandular tissue. SUOMALAINEN indicated that the glands discussed (adrenal and cells of nucleus supraopticus) increase in size both relatively and absolutely during hibernation. BULLARD asked if a concomitant decrease in fat occurs. SUOMALAINEN replied that it does. LYMAN asked if this effect and the neurosecretory activity in hibernation were confined to hedgehogs, and the reply was that it had been investigated also in the golden hamster and found to be true there also. LYMAN asked what time of year these effects were present. SUOMALAINEN replied that it was during hibernation.

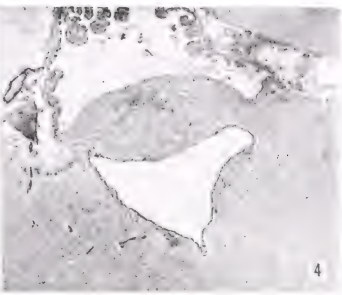
DAWE observed that the nucleus supraopticus lay under the thalamus, which could give credence to the existence of an active "thermostat" in hibernation. SUOMALAINEN said the supraoptic cells are the most probable source of neurosecretory substance produced in the hibernating state. KEHL asked if the appearance of stainable material in the stalk of the hypophysis necessarily indicated active secretion, or rather might

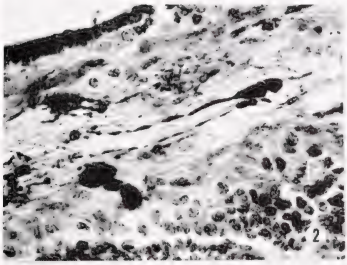
indicate storage with a decrease in secretory rate. SUOMALAINEN said it left this area and passed along the axons of the tractus hypothalamo-hypophyseus into the neurohypophysis for secretion.

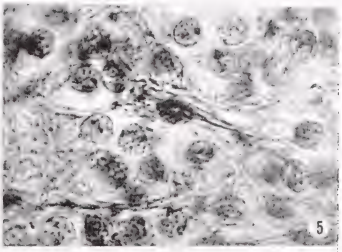
FISHER remarked on the impressiveness of this evidence for specific tissue changes during hibernation. He asked if SUOMALAINEN would be willing to omit the word "stress" in discussing such changes. He pointed out that in working with hibernating animals one must act very quickly, since changes occur very quickly. From the microscopic point of view, one can say that a stress occurs almost instantaneously. Conversely, preparation for hibernation takes a very long period of time before the tissues are ready. This occurs slowly. As a function of time, conditions are thus distorted when one refers to stress changes occurring in hibernation. SUOMALAINEN replied that winter and hibernation, at least in Finland on the north border of the distribution area of the hedgehog, are physiological stressors.

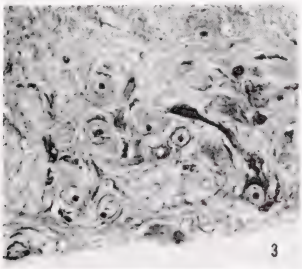
DENYES said that using Bush's technique of analyzing for freely circulating sterols it was found that the amount was halved in hamster blood at cold exposures of 48 hours, and that only trace quantities were present in hibernating hamsters. She thought this was interesting since SUOMALAINEN had found an accumulation of secretory material in the pituitary during hibernation, whereas she had found practically no circulating sterols. These two facts, she believed, indicated that during hibernation secretory material is stored rather than circulated in the blood.











PLATE

Fig. 1. Nucleus supraopticus cells in the hedgehog in autumn. 1125 x. Fig. 2. Herring bodies and intensely stained axons in the hypophyseal tract of the hedgehog in autumn. 1125 x. Fig. 3. Large nucleus supraopticus cells in the hedgehog in early spring. 1125 x. Fig. 4. Cross-section through the subcommissural cell group of the midbrain of the hedgehog. 52 x. Fig. 5. Substance which stains like the neurosecretory substance in the subcommissural cell group of the hedgehog. 1300 x. Fig. 6. Herring bodies in the subcommissural cell group of the hedgehog. 1300 x.

XV

SOME PHYSIOLOGICAL PRINCIPLES GOVERNING HIBERNATION IN *CITELLUS BEECHEYI*

By Felix Strumwasser
National Institute of Mental Health
National Institutes of Health
Bethesda, Maryland

A list of some eight principles governing hibernation in *Citellus beecheyi* is presented and elaborated upon in this paper. In addition I will assert that hibernators did not evolve qualitatively new mechanisms for the manipulation of body temperature and the maintenance of mammalian life systems at temperatures just above 0°C. Instead, hibernation will be considered to involve an extension of already available mammalian regulatory mechanisms for operation at low temperatures.

I. Hibernation in *Citellus beecheyi* Takes Place in Successive Stages with Complete Arousal Between These Stages

Figure 1 is a six-day plot of brain temperature in a partly deafened summer squirrel after it had been in a 7°C environment for twenty days. As can be seen, there are six major dips in the temperature record. Three of them represent the normal temperatures for sleep in the cold in this squirrel, the lows falling between 33.8° and 34.6°C. Three other dips, initiated forty-eight hours apart, consecutively reached lows of 30.8°, 27.9° and 22.9°C. Brain temperature had been continuously recorded during the entire period in the cold and this was the first sign of a phenomenon different in degree and pattern from the usual nightly temperature drops. This pattern of decreasing body temperature to successively lower levels with a complete arousal each time is absolutely typical of all the squirrels studied. Reasons will be developed shortly for calling each of the hibernation drops, as long as they are followed by a lower drop, a *test drop* and each of the minimum temperatures reached a *critical point*. Eventually a final plateau of brain

temperature is reached and hibernation persists. The longest duration of hibernation that I have so far measured in this species, with continuous recording of brain temperature as the criterion, was three days at 6.1° in an environment of 2°C.

Time course of the critical brain temperatures. Figure 2 illustrates the time course of the critical brain temperatures in both winter and summer squirrels. The critical temperatures are plotted at the time of the day at which they were first reached. As a general rule, whether the animal was a winter or a summer animal, hibernation took place with the same pattern. However,

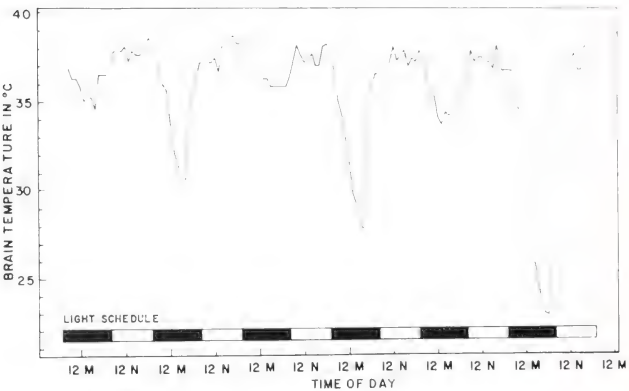


Fig. 1. A 6-day segment of the brain-temperature history of a summer ground squirrel during which time it took its first 3 test drops. Brain temperature plotted every hour. On the time axis midnight and noon are indicated by *M* and *N*, respectively.

during the summer there was a long delay, as long as twenty-six days in one animal, before the first test drop occurred. As can be seen, the relationship between critical brain temperature and time in days after placement into the cold is not linear and the curves of the two winter animals initially parallel each other. The curves of the winter animals are separated in time because one entered hibernation twenty-four hours later than the other; note also that one animal operates on a twenty-four hour hibernation schedule initially (closed circle), and the other on a forty-eight hour schedule (open circle). Because there is

some orderliness in this process I think of these curves as perhaps paralleling the integrated rate of general metabolic preparations necessary for the journey into hypothermia. This is not the only possible process occurring during the preparatory phase. It is tempting to suggest some alteration in brain circuitry or adaptation of synaptic transmission for effective operation at low temperatures as alternative or additional possibilities.

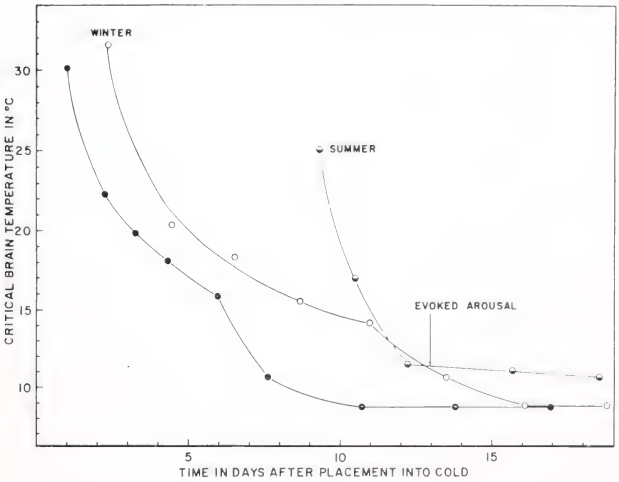


Fig. 2. Time course of critical brain temperatures in winter and summer squirrels. Critical temperatures are plotted at time of day at which they were first reached.

II. Body Temperature Can Be Set and Regulated in This Hibernator Over a Wide Range

Regulation of brain temperature at the critical point. Figure 3 illustrates the kind of regulation of brain temperature observed at three critical points all taken from one squirrel. Note that the strips read from right to left. In *A*, as 31.5°C is reached from a slowly falling temperature, a sharp increase in temperature is initiated rising to 32.1°C; from this peak, temperature again falls slowly, repeating the same pattern as 31.5°C is

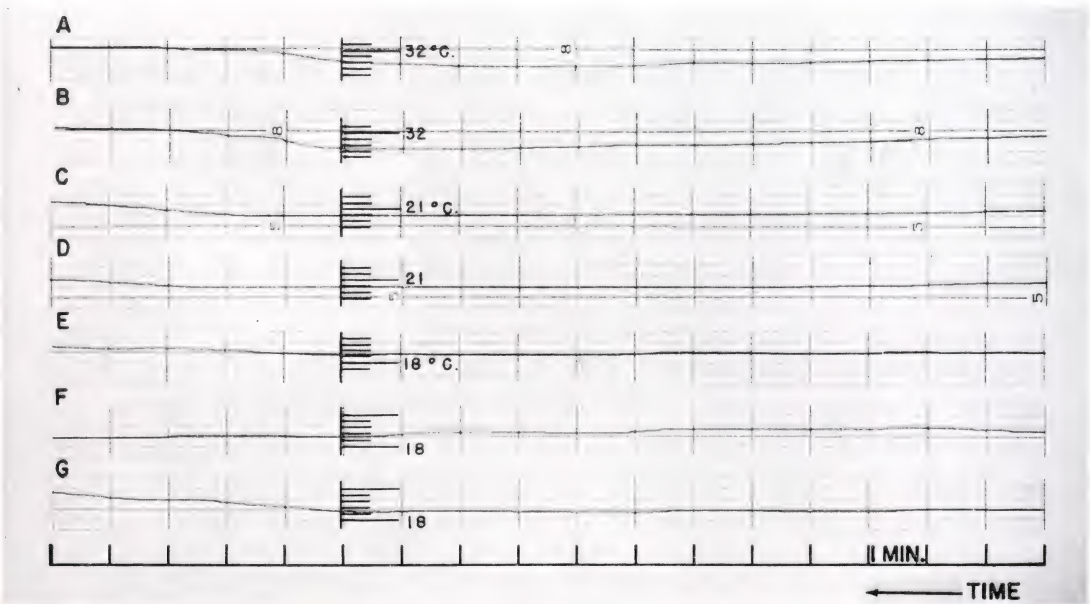


Fig. 3. Regulation of brain temperature at the critical point. In strips *A-G* the *grid lines* indicate 0.2°C. Note time reads from *right* to *left*. *A* and *B*, *C* and *D*, *E*, *F* and *G* are portions of the regulation at 3 different critical points, respectively, in the same squirrel.

reached, as in *B*. Strips *C* and *D* depict the same phenomenon at the lowest temperature arrived at during another test drop in this animal. The regulation in strip *E* is of interest because it consists, in the right half, of oscillations varying between 0.06°C and 0.1°C (see *G* also). Such increases in temperature at the critical point are brought about by shivering. This behavior of brain temperature at the lowest level reached for each test-drop into hibernation — that is, making several passes toward the critical point — is typical in all the squirrels studied. Apparently, the final lower limits of the temperature of each hibernation test drop are regulated and are not allowed to fall below a certain level of the critical point, apparently within 0.1°C and probably less.

Although there are, as can be seen, gaps of the critical brain temperatures in each of the individual cases of Figure 2, when all the animals as a group are examined we find no preferred temperatures and no large gaps in the physiological temperature range. We are forced to conclude that this squirrel can probably maintain and regulate its temperature anywhere between 6° and 39°C.

III. The Timing of Hibernation Can Be Coordinated with the Normal Activity Rhythm

The long wait of the summer animals allowed us to study the timing of the daily temperature rhythm and its relation to hibernation. For example, we followed one individual's brain temperature for twenty-seven days, noting for each day the times that the evening drop and the morning arousal were initiated. Excluding the first seven days in the cold when there was a systematic shift, not to be discussed here, the range of time for the evening drop extended from 4:26 p.m. to 8:06 p.m. It is noteworthy that on the three occasions of hibernation test-drops, the initiation occurred within this range. All arousals were initiated before the light period but perhaps it is more interesting that the arousal from each of the three hibernations was initiated within the normal range of variation, 2:08 a.m. to 7:41 a.m., despite the fall of brain temperature being as large as 15°C in the third hibernation. Figure 4 shows a plot of arousal at what we call preferred hourly intervals. The groups are arranged in order of consecutive time of day; members of a group are arranged in order of consecutive days. As can be seen,

there are several groups separated by approximately one hour intervals.

Where the number of arousals is sufficient to test for significance between the means of adjacent groups (*groups 2, 3, 4* and *5*), the differences are found to be highly significant (*t* test for

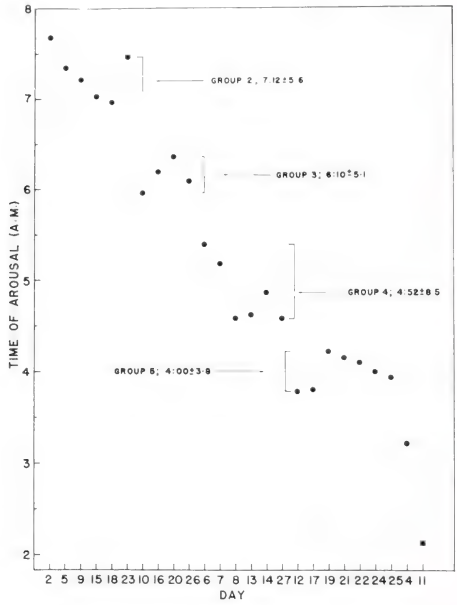


Fig. 4. Arousals at preferred hourly intervals. Groups arranged in order of consecutive time of day; members of a group arranged in order of consecutive days. Limits of *groups 2, 3, 4* and *5* are shown by brackets; mean of each of these groups is given as time of day, with the standard error in minutes.

small samples, $P < 0.001$ in all cases). Note that the first hibernation arousal (day 22) occurred at 4:00 a.m. and the second hibernation arousal (day 24) occurred at 4:06 a.m. amid a group of seven arousals averaging 4:00 a.m. with a standard deviation of 10.1 minutes. The third arousal occurred at 6:06 a.m. which was amid a group of four arousals averaging 6:10 a.m. with a

standard deviation of 10.1 minutes. Apparently arousals, whether they are from test-drops or from normal sleep occur at preferred time intervals, roughly one hour apart while initiation of hibernation test-drops, like the normal nightly temperature drops, occurs randomly within a limited portion of the day.

A time sense which is temperature independent. This animal must then have a time sense most probably due to an internal clock which must be temperature-independent or temperature-compensated since the initiation of arousal most usually anticipates the light period and apparently occurs at preferred hourly time intervals with variation in each class of only a few minutes despite the large decline in brain temperature. However, all arousals are not clock-initiated, at least not all from hibernation. Arousals from test drops are most likely to be since the test drops are short-lasting phenomena. We have seen arousals from deep hibernation (where brain temperature is close to 6°C) that seem to be clearly clock-initiated, because they fall into these preferred hourly intervals, but there are some that are obviously not. Some other internal mechanism is presumably responsible for these arousals.

IV. The Triggering of Entrance into Hibernation is Dependent on the Brain Integrating Three Factors

Three classes of hibernators within rodents. An examination of the information available justifies the recognition within the rodents of at least three classes of hibernators: I, those that wait a variable but relatively long time in the cold — one to three months according to Lyman (1948) for the hamster, *Mesocricetus auratus* — and then enter deep hibernation in one decline in temperature; II, those that do not wait more than a few days and then enter deep hibernation in one decline in temperature, for example, pocket mice, *Perognathus longimembris* (Bartholomew and Cade, 1957); III, those that wait only a few days but never enter deep hibernation in one decline, going through a series of consecutively lower temperatures and arousing completely in between hibernation drops. Besides *Citellus beecheyi*, this group probably includes *Citellus tridecemlineatus* (Johnson, 1931; Foster, 1934; Zalesky, 1934), apparently the dormouse *Myoxus glis* (Wyss, 1932), probably the marmot *Marmota marmota* (Dubois, 1896), the woodchuck *Marmota monax* (Rasmussen, 1916; Benedict and Lee, 1938), and possibly *Citellus parryi* (Musacchia and Wilber, 1952; Svihla and Bowman, 1952).

Critical points, test drops and preparations. The difference between these groups may lie only in the fact that the first waits until it is fully prepared before entering hibernation, the second may be virtually prepared at all times or needs very little preparation, while the third group begins test drops while preparations are in progress and goes only as far as the preparations have gone. *Certainly the fine regulation of the low temperature reached at each entrance is suggestive that it is rather critical to the squirrel not to go below it for that day.* This is the reason these are called critical points. It is proposed that at each

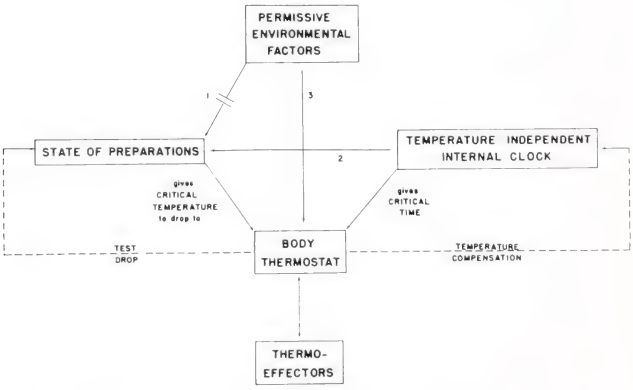


Fig. 5. Three-factor theory of hibernation.

drop there is some testing of how well preparations have gone, so for the time being these drops before the final plateau are labeled test drops.

A three-factor theory of hibernation. Figure 5 summarizes our findings, so far, in a diagram which explains, or attempts to, how the triggering of hibernation might be accounted for. There are three main factors which are necessary to account for the time when the thermostat is turned down.

a. *Permissive environmental factors.* When environmental factors are "permissive," biochemical preparations for hypothermia proceed. Lack of adequate environmental factors may result in the preparations not proceeding at all (*1*, Fig. 5). Both internal and external environmental factors are important

in this concept. One would guess that a serious competitor for hibernation would be reproductive drive, even in the presence of permissive external factors; the latter requirement may be different from species to species and would consist of factors such as noise, terrain, level of environmental temperature, food supply and so on.

b. *State of preparations.* The preparations are considered to be biochemical and necessary for survival of the total animal under conditions of prolonged hypothermia. The body thermostat, by being kept informed of the state of preparations, can pull the trigger for hibernation if these are sufficiently under way by the correct time of day.

c. *Temperature independent internal clock.* The clock allows the animal to synchronize the various stages of hibernation with its normal species-specific activity schedule in the field and for this reason is of considerable survival value. If the preparations have reached a certain stage and have done so by a certain critical portion of the day and there are no interfering environmental factors (*3*, Fig. 5), the thermostat activates the adequate thermoeffectors and the animal at this time is said to be entering hibernation.

Some requirements for central (brain) integration of the three factors. Whatever biochemical processes preparatory to the journey into hypothermia are proceeding in the cold environment, they must exert an influence on the temperature regulating mechanisms, since this mechanism seems to be able to compute just how cold it can permit the animal to become. It is also conceivable that through some feedback mechanism the state of preparations is informed of just how well the body machinery is performing at each of the critical temperatures and can make the appropriate adjustments, if needed, for the next drop (left dotted line, Fig. 5). Evidence for this may possibly come from the observation that each succeeding entrance into hibernation occurs at a faster over-all rate (Fig. 6). Additional flexibility of the system may be gained if the preparations may be speeded up (or slowed down) depending on the time of day, (*2*, Fig. 5).

Another feedback mechanism allows the internal clock to be informed of the temperature/time characteristics of the drop: this would give the clock, by a mechanism of computing the necessary compensation for temperature effects, its temperature independent appearance (right dotted line, Fig. 5).

To sum up, the squirrel is rather particular as to when it drops its body temperature; preparations must be at some critical

level before it will do this and when it takes its initial drops it will go only as far, apparently, as these preparations safely allow it to go.

V. The Curves of Cooling/Time During Entrance into Hibernation are Under Close Control and Represent Something More than Passive Cooling

Form of temperature curves of consecutive entrances into hibernation. Figure 6 is a plot of four consecutive entrances into hibernation from the same squirrel, terminating at four

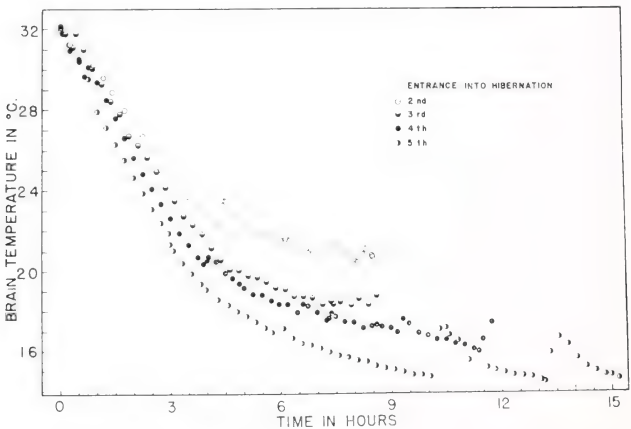


Fig. 6. Form of temperature drop during 4 consecutive entrances into hibernation. Zero time is time at which brain temperature reached 32°C in all 4 curves. Brain temperatures plotted at least every 15 min.

successively lower critical points. Zero time for the four entrances was taken as the time when the squirrel's brain temperature had reached 32°C, since, as a general rule, once this temperature is reached there never occurs a spontaneous turning back.

It can be seen that each time the squirrel enters hibernation it drops its temperature a little faster than the previous time so that by the fifth entrance (series began at entrance 2) at 24°C the squirrel was 20 minutes ahead of the fourth entrance, 45 minutes ahead of the third entrance, and 65 minutes ahead of the second entrance. It will be noticed that by 28°C there is

already an obvious separation of the four curves. All four curves, as is the general rule, have a higher virtually linear rate of decline in the initial limb of the curve, followed by a decrease in rate until a plateau is reached.

There is a very significant feature in these curves that should be noted. Although the decline in the second limb of each curve is exponential-like, the animal is not bound to this relationship. After two rapid temperature increases in the fifth entrance curve (as the critical point was being approached), temperature each time declined with a steep slope very close to that of the initial limb. *This immediately suggests that the gradient between core and air temperature is not the only factor in determining the shape of the cooling curve.* Shivering as detected electrically is responsible for the shape of the second limb of the curve.

Significant details of expanded temperature curve. Figure 7 shows actual records of portions of the four consecutive entrances into hibernation referred to in Figure 6. First, it can be seen throughout the records that there are plateaus and gentle steps of declining temperature. The steps are not of uniform amplitude or rate, nor are the plateaus shown of uniform length. These three aspects of the plateaus and steps depend on the portion of the temperature curve studied and which of the series of entrances is being examined. For example, the two steps (*A* and *B*) in entrance 2 are small and have very gentle slopes. Compare this to the steps in entrance 5 (particularly *L* and *M*) which have considerably steeper slopes. In general it has been found that the later entrances of a series have on the average shorter plateaus, and greater amplitude steps or faster rates of decline of temperature within the step or a combination of these last two factors. It is these factors apparently which explain the faster over-all rate of temperature decline in consecutive entrances. As the critical point is approached, plateaus become longer. Small oscillations of temperature — a rising and falling of about 0.1°C — become apparent (strip *F*) and the amplitude of the steps is quite small (strip *K*), approaching the dead band of the potentiometer recorder. Maximum slopes of some of the steps are in the neighborhood of 0.3°C/min. (for example, strip *G*).

Heat-production and heat-loss mechanisms. During entrance into hibernation, the squirrel intermittently shivers as detected by the chronic muscle leads (Fig. 11). Figure 8 is a plot of brain temperature at 1-minute intervals as related to the actual record

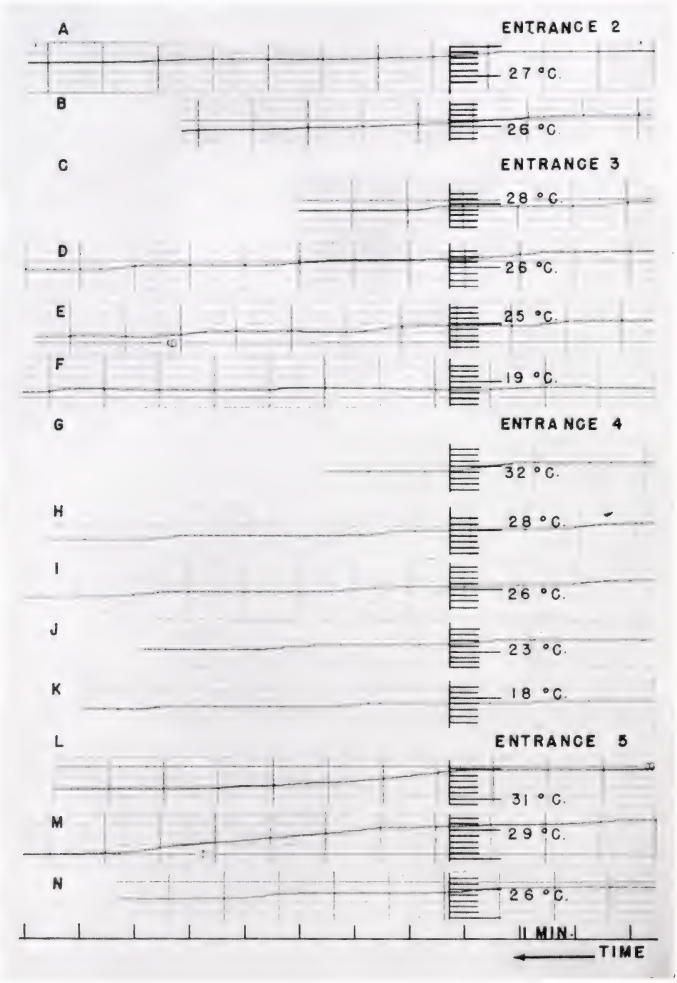


Fig. 7. Steps and plateaus in declining brain temperature curve during entrances into hibernation. Strips selected come from the 4 entrances into hibernation, completely portrayed on a compressed time scale in Figure 6. Temperature graduations are 0.2°C. Time reads from right to left.

of surface skin temperature obtained by a permanently implanted thermistor. The initiation and length of shivering is illustrated by the solid bar while on the line below the hatched area represents the presence of continuous small amplitude muscle activity — muscle tone — and the clear area its absence.

If one follows the graph through, it will be seen that the following phenomena occur: 1) shivering during the step down (*A*, *B*, *C*); 2) shivering during the plateau after the step down

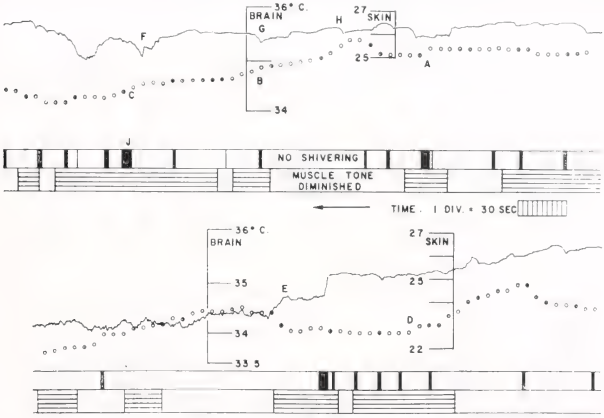


Fig. 8. Heat-loss, production and conservation mechanisms during entrance into hibernation. Record of skin temperature (thermistor) is continuous but brain temperature (thermocouple) is plotted every minute (*open circles*). Below, the *solid bars* indicate shivering while the horizontally *hatched bars* indicate the maintenance of muscle tone. Time reads from right to left.

(*D*); 3) a fall in temperature of the skin concomitant with a rise of temperature in the brain when there is no shivering (*E*); 4) when brain temperature falls, there is a rise of skin temperature preceding the drop in the brain with a return to original temperature, the major peak of this double wave lasting about 5 minutes and having an amplitude of 0.8°C (*F*); 5) where only somewhat late low amplitude increases in skin temperature occur during a fall in brain temperature (*H*), muscle tone and shivering are absent.

We cannot be at all precise about the time relations since the error in producing a graph on such a slow time scale from different recorders may be as large as 15 seconds (about the size of the open circles). However, about 2½ minutes after the start of a shivering burst lasting 1 minute a plateau in brain temperature is obtained (*J*). The rise in skin temperature occurs ¼-1 minute before a step down in the temperature of the brain.

Shivering as a brake during entrance into hibernation. The fact that the fine detail of the decline in brain temperature consists of long plateaus and shorter steps means that some time is being spent without any temperature change. Three-tenths degree centigrade per minute represents a normal rate of decline of temperature during a step down in the later entrances. If it could be continued at this rate, the squirrel would be able to drop 30°C in 100 minutes. Dropping this much takes a squirrel entering hibernation about 18-22 hours. The production of plateaus as initiated and maintained by shivering is responsible for this slowing of the temperature decline. Shivering then acts as a brake slowing down the rate of fall of temperature as the animal enters hibernation.

Mechanism of the "step down." The mechanism of the declining steps in brain temperature appears to be a vasodilation on the skin of the back of the animal, which is exposed out of the nesting material to the cold air, and a reduction of muscle tone during steep drops, but with the maintenance of muscle tone and actual shivering in drops which are less steep. Apparently a rise in brain temperature can occur without shivering by vasoconstriction on the skin of the back. All in all, there appears to be a coordinated interplay between the cooling powers of the skin and its ability to reduce heat loss and the heat production capacity of shivering and maintenance of muscle tone. By controlling these processes, the animal entering hibernation can control the decline of temperature rather precisely. The physiological significance of avoiding a rapid cooling is not known but apparently the squirrels studied never do otherwise. We feel, for reasons discussed elsewhere (Strumwasser, 1959), that Lyman and Chatfield's (1955) suggestion, "that the decline in metabolic rate is the cause of the decline in body temperature," is an oversimplification. An active inhibition of general metabolic rate during entrance into hibernation seems quite unlikely from the fact that rapid alterations of deep body temperature are possible and furthermore appear to be under fine control (Figs. 3, 6, 7, 8).

VI. The Heart-Rates During Entrance and Arousal Follow Different Paths with Respect to Temperature

When heart rate is plotted for several hours preceding and during entrance into hibernation (Fig. 9), it is obvious that there is a striking decline, as the hibernation drop becomes continuous (occurring between 34.2° and 33.6°C), from the rate of 153 beats to 68 beats/min.; that is, a decline of more than one-half the rate in 30 minutes with a lowering of temperature during

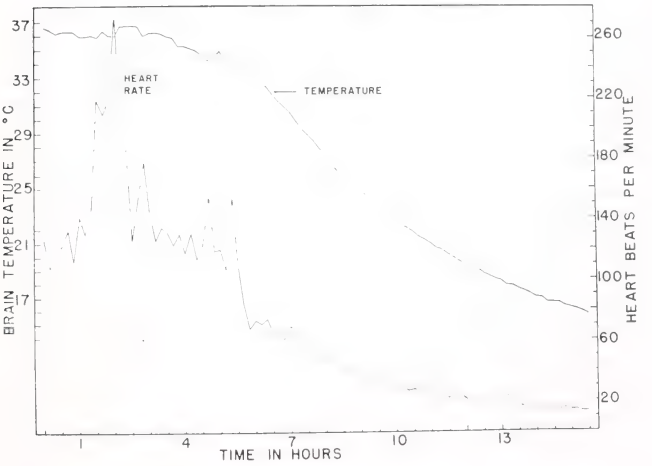


Fig. 9. Heart rate during entrance into hibernation. Heart rate and temperature plotted every 10 min.

this time of only 0.6°C. The decline in heart rate is rather more undramatic though, if one considers that rates as low as 108 beats/min. may occur at a time when brain temperature is fluctuating between 35°-37°C several hours prior to the act of entrance.

When the heart-rate/temperature relation is analyzed in the same squirrel during a consecutive entrance and arousal (Fig. 10), it can be seen that the curves are fair mirror images of each other, together having the appearance of a hysteresis loop. For arousal, heart rates as high as 246 beats/min. may occur

at brain temperatures as low as 16°C, while during entrance, rates as low as 84 beats/min. occur at temperatures as high as 34°C.

The mirror-image-like relation of the heart-rate/temperature curves for the squirrel arousing from and entering hibernation suggests that the heart is being driven to either side of the Arrhenius constant which would be obtained from the denervated or isolated heart, with strong excitation during arousal and strong inhibition during entrance. Dawe and Morrison (1955) who used hedgehogs and two species of ground squirrels

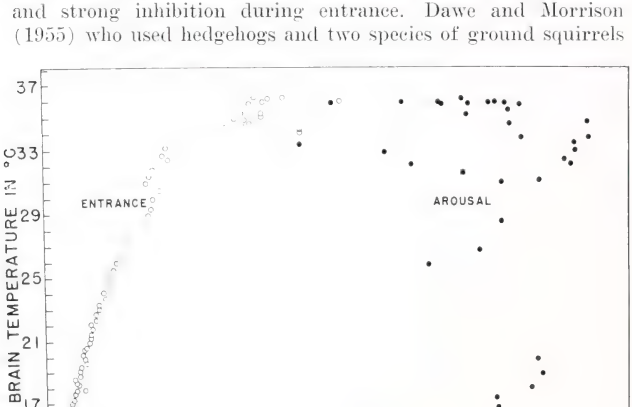


Fig. 10. Heart rate/temperature relation during a consecutive entrance into and arousal from hibernation.

for their study reported somewhat similar results. This suggests a dominant sympathetic influence on the heart during arousals, and during entrance a predominantly parasympathetic one.

One should note however, that the Q_{10} between 23.5° and 17°C (critical point of this drop was 12.5°C) in the animal entering hibernation falls between 3 and 2 which would indicate relative temperature dependence for the heart in this region. Prior to this range the active inhibition of heart-rate might be associated with the diminished oxygen needs of a very relaxed musculature (probably beyond that present with normal sleep).

VII. The Spontaneous Electrical Activity of the Brain is Maintained During Entrance Into and Maintenance of Hibernation Despite Declining and Low Temperatures

Some neural correlates during entrance. So far brain-wave records taken from squirrels hours before entrance into hibernation have revealed no signs of specific activity by which the entrance could be predicted. The appearance of excessive spindling in most areas of the cortex occurs at a time when the temperature is already dropping. However, these are intermittently broken up by desynchronizations lasting as long as 3-5 minutes during which time temperature is stable and may be associated with movements of the squirrel in its nest and/or periods of shivering. As soon as brain temperature declines below approximately 34°C, (the normal nightly sleeping temperature) several interesting electrical patterns appear with rather specific interrelations.

Figure 11 illustrates localized patterns of activity within the lateral motor cortex, the basolateral amygdala and the dorsal hippocampus. The specific discharges are centered around shivering which is recorded on the fourth channel together with the activity of the right sensory cortex.

Motor cortex. Note in strip *A* of Figure 11 (occurring during a plateau of brain temperature at 23.4°C) the grouping of a specific 9-10 cps rhythm in the motor cortex growing in amplitude and continuity until apparently as the peak amplitude is reached shivering takes place. Compare the activity to that in the sensory cortex which shows only a nonspecific spindle burst close to shivering (strips *A* and *C*). Note the continuation of the 10 cps rhythm in the motor cortex throughout shivering and for several seconds after shivering. These 1-2 second bursts of 10 cps activity start to build up some 4-9 seconds before shivering but low amplitude 10 cps activity in the form of waxing and waning bursts seems to be ever-present during entrance into hibernation and is not as apparent at other phases of the hibernation or sleep cycle.

Strips *C* and *D* (occurring during a plateau at 21.4°C) repeat an illustration of the points made but are presented to show the long 10 cps after-shivering discharge present in the motor cortex concomitant with the maintenance of muscle tone (compare the thickened line in channel 4 strip *D* with the initial part of *A* and the terminal part of *B*).

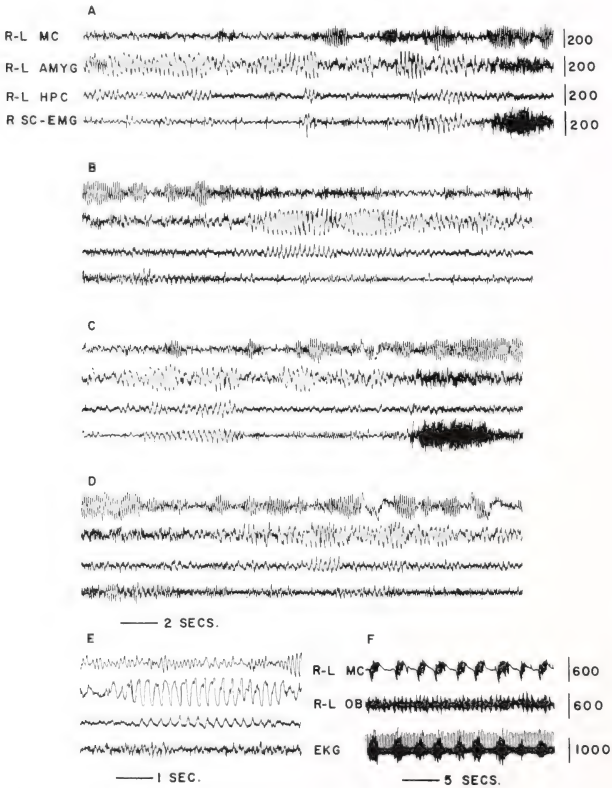


Fig. 11. Activity in the brain correlated with shivering during entrance into hibernation. *A* and *B*, *C* and *D* are continuous strips. *E* is a faster record showing the degree of synchronization between the hippocampus and amygdala during the 5 cps burst. *F* is from a different squirrel which had a positive motor cortical lead but shows the difference in the activity of the motor cortex during arousal shivering. Calibration is in μv. *AMYG* — amygdaloid nucleus; *HPC* — hippocampus; *L* and *R* — left and right sides of brain, respectively; *MC* — motor cortex; *OB* — olfactory bulb; *SC* — sensory cortex.

Amygdaloid-hippocampal interrelations. A rather constant finding after shivering as the motor cortex activity dies is the appearance of high amplitude 5 cps rhythm in the amygdala during which an identical rhythm in the hippocampus breaks out. Strip *E* is a faster record to show the fairly close synchrony between these two areas; note that the hippocampal rhythm appears in the midst of the amygdaloid burst which outlasts the hippocampal activity.

Absence of these specific correlates during arousal shivering. It is estimated that around 500 shiverings were recorded in all the squirrels with some or all of the three positive brain leads; the interrelated pattern of the rhythms described was always present with each shivering, and at no other time. These patterns are not seen with the shivering present at other stages of the hibernation cycle.

Interpretation of the specific EEG patterns. The motor cortical discharge may well be the sign of a facilitative downstream discharge which initiates or aids in the development of shivering; at any rate this is a suggestion based on the long latency before shivering begins. The amygdaloid-hippocampal rhythm is mostly apparent when the motor cortex 10 cps discharge is at its low or absent or beginning to disintegrate (strips *A*, *B* and *C* of Fig. 7). In *D*, where the motor cortical discharge takes quite long to dwindle down, the amygdaloid pattern is present but is not as large as in *B*. It is interesting to note that when this occurs the hippocampal rhythm is weak and the synchrony not as good. For these reasons it is felt that the amygdala and hippocampus are involved in a mutually inhibitory mechanism with the motor cortex or something preceding it but are not involved at the level of the fundamental shivering mechanism. Both excitatory and inhibitory influences from the hippocampus and amygdala on the autonomic aspects of mammalian activity have been demonstrated by numerous investigators, in particular Kaada (1951). It is well known that the motor cortex is not necessary for shivering in non-hibernating mammals during a temperature stress (Isenschmid and Schnitzler, 1914) and it is presumably not involved in shivering in the ground squirrel at other times, but it is clearly correlated with shivering during entrance into hibernation. This suggests that a hierarchy of brain control systems is probably exerting its influence on the fundamental system responsible for shivering; that is, these "higher" systems are determining the initiation and termination of shivering and its integration into the coordinated pattern responsible

for the precise form of the curve of the temperature drop. This may indeed be a general phenomenon in animal behavior; similar activities in the same animal may be brought about by different hierarchical systems, each system complete within itself and organized to accomplish a particular goal.

VIII. During Deep Hibernation Certain Complex Regulations and Behavior are Compatible with a Striking Decline in Cerebral Electrical Activity

Behavior during deep hibernation. Postural adjustments. During maintained hibernation at brain temperatures as low as 6.1°C the deafened hibernator is not completely motionless.

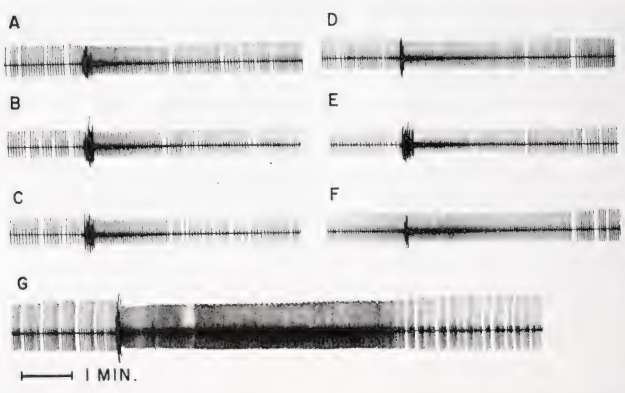


Fig. 12. Heart rate during a series of postural adjustments. Brain temperature remained constant throughout at 10.7°C. Darkened burst of muscle activity is synchronous with a postural adjustment.

At this temperature there are about two or three somewhat periodic postural adjustments per hour. This in its simplest form consists of a slow raising of the head and forepart of the body with a gentle return to the original position. However, there can be more complex coordinations in this behavior. After raising its head and the forepart of its body, the squirrel may slowly shift its position in the nest several degrees by properly orienting the

forepart of its body before gently returning to its nest. During these postural adjustments, cardiovascular compensations occur. As can be seen in Figure 12 heart rate increases. Not infrequently there is a clear-cut increase in heart rate anticipating the postural adjustment (strip *F*). Note the striking rebound inhibitions present after a particularly long duration of accelerated heart-beat in *G*.

Response to sound stimuli. The ability of three undeafened, unimplanted deeply hibernating squirrels to respond to various sounds in their environment has been studied. Oral temperatures have been measured from a few minutes to an hour after termination of the particular experiment. Upon tapping sharply two or three times on the metal top of the aquarium or on one of the glass panes, one first observes slow uncurling of the pinna if it is relaxed against the side of the head. If the tapping is repeated (sometimes it takes 1 or 2 more presentations), the squirrel invariably cocks its head, directing one ear toward the source of the sound by slow rotation of the neck with or without raising the forepart of its body. Even if the tapping is not presented for the next 20-30 minutes, the pinna remains erect and the head remains in the same position or may be slowly turned in the opposite direction after a variable lapse of time. Eventually, the head is rotated to its original position and the animal continues to hibernate as judged by respiratory rate and behavior for at least 2 hours, a time during which most of the arousal is normally accomplished. An oral temperature of 5.8°C in an environment of 2.1°C was the lowest temperature recorded within 20-30 minutes of this sequence. If vigorous tapping is maintaind for a few minutes an arousal is invariably initiated.

Vocalization in deep hibernation. When a squirrel in deep hibernation is touched, one of the first responses is a sustained loud shriek lasting about 0.55 second (Fig. 13) after which, of course, an arousal is initiated. There are differences between the hibernation vocalization and the normal squirrel chatter in pattern and in length although it is interesting, as high speed analyses have shown, that there are no conspicuous differences in the frequency range of the notes. Peak frequencies for the vocalization of the hibernating squirrel are in the neighborhood of 3200 cps at 10.7°C while for the normal squirrel chatter at 37.2°C they are 3750 cps.

Focusing of attention, discrimination and localization of the stimulus source. The fact that the squirrel has an auditory-pinna

reflex and adjusts the position of its head toward the source of a sound stimulus is evidence for the ability of the animal to focus its attention and attempt to locate in space the source of the sound. The fact that squirrels do not awake to this sound unless it is maintained for at least a few minutes is evidence for discrimination.

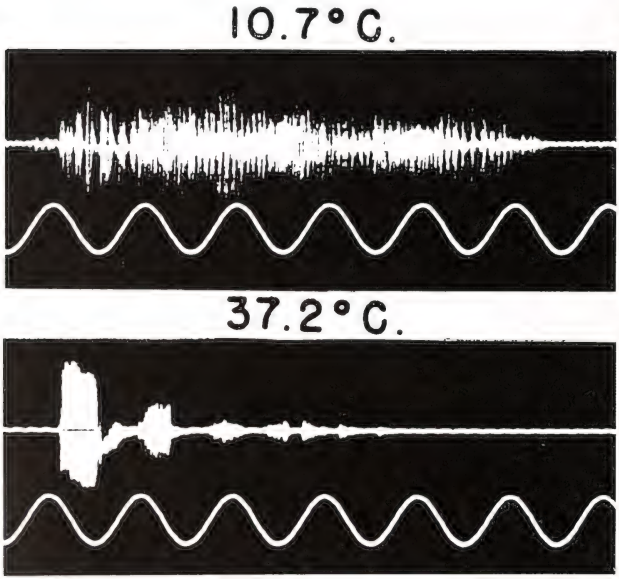


Fig. 13. Vocalization pattern of a hibernating (*upper*) and non-hibernating (*lower*) disturbed squirrel. Vocalizations were tape recorded and later played back into a cathode-ray oscilloscope and photographed. Temperatures indicated are cerebral; time line displays a 10 cps sinusoidal oscillation.

If this discrimination is not sufficiently sophisticated, consider Mullally's (1953) observation that squirrels (*Citellus lateralis*) often hibernated in the presence of active members of the same species: "At times, with four in a box, two were hibernating and two were not. Although scuffed by the active ones, the hibernating individuals did not awaken until they were

handled'' (by the experimenter). This observation suggests species-discrimination in an animal whose core temperature was probably below 10°C (see his Table II for hibernating rectal temperatures)! All these behavioral phenomena during deep hibernation are of great interest, in particular when the electrical activity of the brain at these low temperatures is examined.

Regulatory mechanisms and brain waves in deep hibernation. Brain temperatures and heart-rate during deep hibernation. After the final critical point is reached (see Fig. 2) brain temperatures remain constant for prolonged periods of time (10 hrs

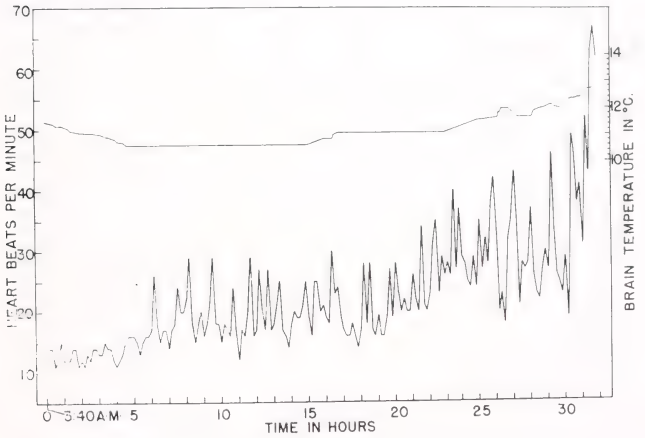


Fig. 14. Heart rate and brain temperature during maintained hibernation. Heart rate and brain temperature plotted every 10 min. Brain temperature plotted at shorter intervals during changes.

to 68 hrs, with later entrances having longer maintained plateaus) with no change in temperature within ±0.05°C. Figure 14 is a plot of heart rate and temperature over 32 hours as a squirrel approached, maintained and aroused from its final critical point for the first time. During this time environmental temperature fluctuated between 7-8°C. It can be seen that during the maintained low brain temperature of 10.7°C, both the average heart rate and amplitude of oscillations of heart rate are

greater than the period during which brain temperature approaches the critical point. This is a remarkably constant feature of such plots. It suggests that the relative level of sympathetic tone is increased at this lower level of temperature.

An increase in temperature from the critical point to a higher plateau before an arousal (defined as the point from which a continuous rise without any decline in temperature occurs, such as at 29.75 hours after 0 in Fig. 14) is often seen, but there are cases when it is absent. Note the continuous increase in average heart rate during the higher plateau and before the next

Fig. 15. Intermittent initiation of motor unit discharge during deep hibernation. Strips *1* to *7* and *8* to *14* are continuous. The small unit in strip *7* stopped its discharge 12 sec after the end of the strip; 3¼ min elapsed between strips *7* and *8*. Brain temperature maintained at 8.8°C.

rise. The increase in heart rate prior to a spontaneous arousal is in agreement with the observations of Dawe and Morrison (1953) and comparable to the increase seen in the heart rate before the temperature rise of an evoked arousal in the hamster (Chatfield and Lyman, 1950).

Muscle tonus during deep hibernation. During these prolonged maintained low temperatures, tonus is present in muscle. Figure 15 is a typical continuous record of two muscle units with a

pause of 3¼ minutes between strips *7* and *8* recorded while brain temperature was maintained at 8.8°C. The dark bursts of activity are respirations while the larger, longer duration deflections are heart beats. It will be noted that the large unit begins firing at relatively high frequency (4/sec) near the middle of strip *1*. Not until near the start of strip *4* (some 45 secs later) did the second smaller unit start firing, quite obviously out of phase with the first and outlasting it. The small unit did not stop firing for another 12 seconds after the end of strip *7*. As strips *8* to *14* show, the cycle may be initiated and completed the very next time with only the large unit firing.

We have had cases where five or six units were identifiable in the record. These have been analyzed to determine which unit starts the cycle, the order of appearance of the units, the length of the cycle, and the silent interim, over continuous periods as long as 12 hours. No simple formula or pattern is apparent and, to date, it has not been possible to predict which of the units will initiate the cycle or how many will come in, or the order of their appearance. We also find that there is no obvious relation to any of the oscillations of temperature measured on the surface of the skin. The phenomenon included long periods of silence, as long as 5 minutes. More often than not, there is seen a clear cut specific discharge in the motor cortex before the muscle units appear in the record, with maintained but lower amplitude fast activity lasting a variable period of time (strip *A*, Fig. 16, brain temperature at 6.1°C). The maintenance of muscle tone in deep hibernation is extremely interesting in view of the low cerebral temperatures during this state. The phenomenon of periodic initiations of unit discharge with units appearing in apparently random order and phase relations each time may be considered the resultant of a central switching mechanism. The "central switching mechanism" appears to be operating quite normally despite cerebral temperatures as low as 6°C.

Brain waves in deep hibernation. Brain wave activity is far from absent even at temperatures as low as 6.1°C. Figure 16, strip *A*, demonstrates activity in the motor cortex, medial preoptic area, septum, and the ventromedial nucleus of the hypothalamus, and relative silence in a subcortical sensory area, the lateral geniculate body. Note the discharge in the septum. It is quite coincidental to the activity of the cortex and presence of muscle units but it is a rather typical example of a pattern, in this instance seizure-like, unique to each particular electrode location, which in deep hibernation periodically repeats itself.

Fig. 16. Brain wave activity during deep hibernation; and its suppression by pentobarbital. Strip *A:* brain temperature at 6.1°C; Strips *B* to *E* from another squirrel, in *B* at a brain temperature of 10.7°C; Strip *C:* 1¼ min after touching the squirrel with a glass rod; Strip *D:* 5 min after an intrathoracic injection of pentobarbital; Strip *E:* 15 min after *D*. *L* and *R* — left and right sides, respectively; *LG* — lateral geniculate; *MC* — motor cortex; *MPO* medial preoptic area; *MRF* — mesencephalic reticular formation; *SEP* — septum; *VMH* — Ventromedial hypothalamic nucleus. Calibrations in microvolts.

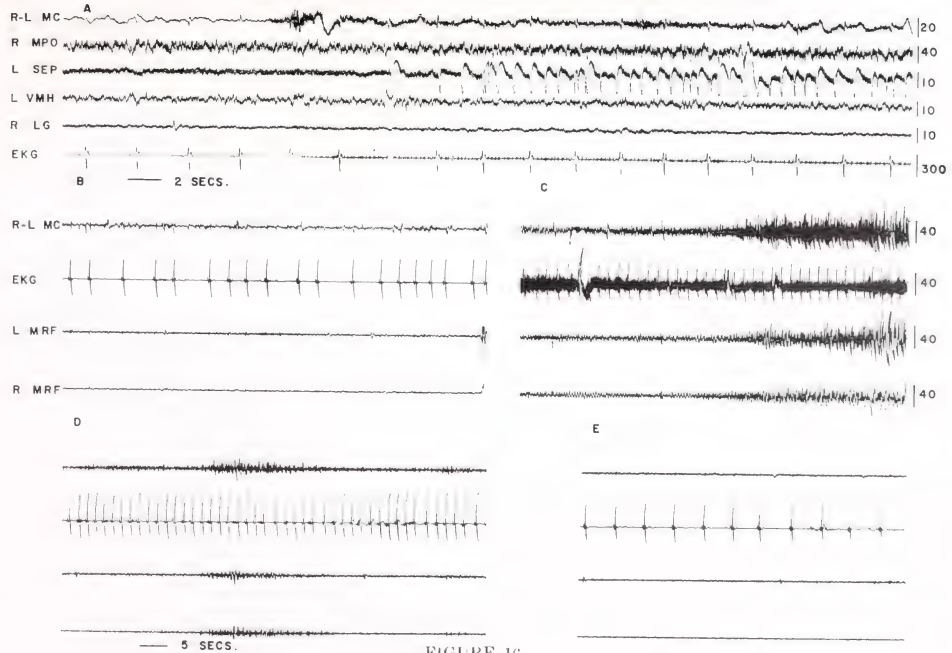

A
R-L MC
R MPO
L SEP
L VMH
R LG
EKG
20
40
10
10
10
300
B
2 SECS.
C
R-L MC
EKG
L MRF
R MRF
40
40
40
40
D
E
5 SECS.

FIGURE 16

We have never seen persistent silence in any brain area during maintained deep hibernation. If one examines long term EEG records, there is always, in the most inactive cases, some patterned activity which repeats itself.

Figure 17 displays this principle in a purely sensory area, at a brain temperature of 10.7°C. Strip *A* shows a 4-6/sec. rhythm building up in the right and left olfactory bulb with the appearance of a similar rhythm in the dorsolateral preoptic area no more than 0.5 mm ventral to the anterior commissure. Respirations (occurring at every shortened cardiac cycle) tend to break up the rhythm momentarily but do not initiate the typical slow wave and 50 cps activity seen in non-hibernating squirrels.

When simultaneous records of each bulb relative to an indifferent grounded point are analyzed (strips *B-F*) it is seen that several phenomena occur: *a*) The right bulb may start this discharge first and as it builds up a similar rhythm can be seen starting in the left bulb at a time when activity begins to appear under the electrode close to the anterior commissure (strip *E*). *b*) The left bulb may start the discharge first and the right then follows at a time when activity in the commissure appears (strip *F*). Note that some of the waves in this latter channel are predominantly up and some predominantly down, indicating perhaps communication through the commissure first in one direction and then the other. *c*) The left bulb may start firing without a discharge in the right, or vice versa, even though similar rhythmic activity appears in the commissure (strip *C* shows the left, strip *B* the right). *d*) Sometimes the right or left bulb may show a small isolated burst without any apparent activity in the commissure (strip *D*).

Effect of pentobarbital on brain wave activity in deep hibernation. Such bursts are wiped out within 10 minutes after an intrathoracic injection of pentobarbital, 30 mg/kg (¾ the dose necessary to anesthetize a non-hibernating squirrel for 1 hr.).

Strip *B* in Figure 16 is taken from a squirrel with a brain temperature of 10.7°C. Note the relative silence of points in the right and left mesencephalic reticular formation. Strip *C* is the same squirrel; the strip begins 1¼ minutes after touching the squirrel with a glass rod. Activity immediately appeared in the relatively quiet areas, the heart speeded up and continuous muscle activity was initiated.

Strip *D* occurs 5 minutes after 30 mg/kg of pentobarbital was injected intrathoracically. Heart beat, muscle activity and brain activity are obviously declining. Strip *E* occurs 15 minutes after

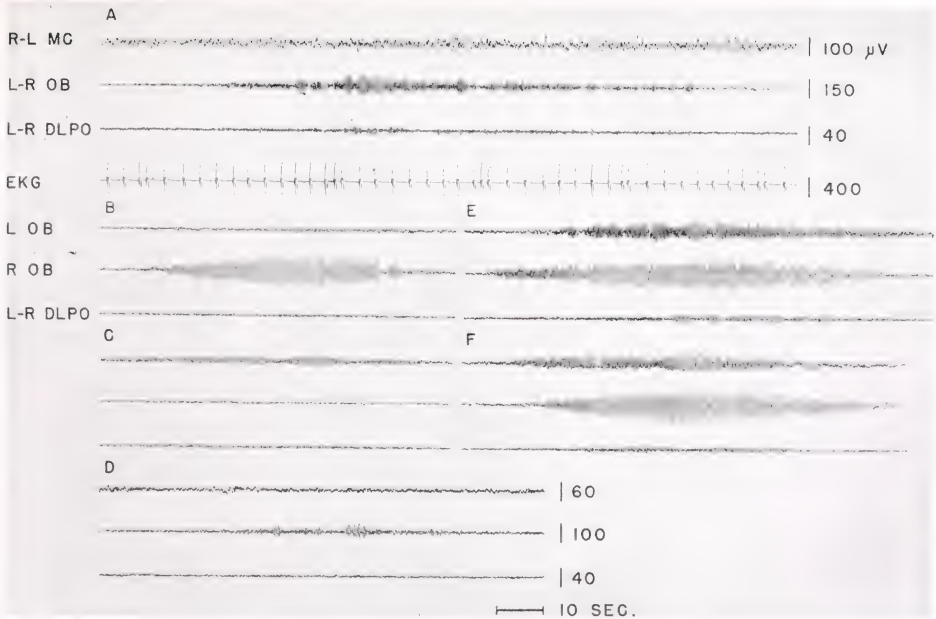


Fig. 17. Study of discharges in right and left olfactory bulb during deep hibernation. Brain temperature, 10.7° C throughout. *DLPO* — dorsolateral preoptic area; *L* and *R* — left and right sides of brain, respectively; *MC* — motor cortex; *OB* — olfactory bulb.

D. Whatever fluctuations were present from now on in the brain channels remained even after the heart eventually stopped some 10 hours later and were indistinguishable from the noise level of this particular instrument. The animal stopped its respiration 35 minutes after the anesthetic was given. In one more squirrel injected with 40 mg/kg of pentobarbital, the same sequence of events occurred. After a preliminary activation due to picking up the animal, all activity present declined to a level indistinguishable from the instrument noise level even though a good deal of maintained spontaneous activity was present prior to picking up the squirrel. The heart beat, however, lasted 13 hours before total failure.

Spontaneous activity in the brain of the hibernator. According to Lyman and Chatfield (1955) in a review on hibernation, ". . . in deep hibernation, the cerebral cortex of the hibernating animal shows no spontaneous electrical activity, but the species vary in the temperature at which electrical activity ceases." At the lowest critical points, 6.0° and 6.1°C in two squirrels, spontaneous activity was consistently seen (as these 2 animals together re-entered hibernation a total of 6 times) in the motor and sensory areas of the cerebral cortex. Lyman and Chatfield's statement is based partly on their own research on acutely measured electrocorticograms of arousing hamsters (Chatfield *et al.*, 1951) and measurements made on one woodchuck (Lyman and Chatfield, 1953) in deep hibernation with two implanted cortical electrodes. Apparently they used low and constant amplification in all published records, even though temperatures fluctuated between 7° and 18°C for the woodchuck and 11.4° and 29.4°C for the arousing hamsters.

It should be emphasized once more that there is a good deal of spontaneous activity present in a variety of cortical and subcortical sites during deep hibernation in *Citellus beecheyi* (Fig. 16) but sufficient amplification must be used. Even when "silent" areas of the brain are observed they are not persistently silent. Long term records with or without the presence of the investigator in the room have shown periodic bursts of activity, sometimes generalized, however mostly independent and unique and repeatable in pattern for each area without any change in cerebral temperature. It is interesting to note that Lyman and Chatfield (1953), despite their already-stated conclusion, observed for the one marmot studied, "slow nondescript, spontaneous cortical activity" at 7°C, "although it was sporadic."

In the records from Kayser's group (Kayser *et al.*, 1951; Rohmer *et al.*, 1951) on cortical activity during deep hibernation in the European squirrel, *Citellus citellus*, the same phenomenon is apparent but it is not clearly stated in their two papers whether the attachment of leads to the squirrel in their system always initiated an arousal or not; apparently they never recorded in the cortex from animals entering hibernation.

The analysis of discharges during deep hibernation in the two olfactory bulbs and its interconnection suggests some of the factors involved in these intermittent discharges; it is assumed that the activity recorded in the third channel is volume conducted from the ventral border of the anterior commissure at which the electrode was aimed. An anatomical factor involved in these discharges is the symmetrical interconnecting arrangement of the brain allowing for activation of one side if the other is discharged by some means. Of the physiological factors, apparently a certain level of activity is necessary before transmission out of a particular bulb can occur (*D*, Fig. 17) and even when transmission to the other bulb occurs there may be no activation of the discharge (*B*, *C*, Fig. 17), a fact probably indicating that the elements responsible for the discharge in the contralateral bulb are fluctuating in their excitability due to intrinsic or extrinsic causes.

Significance of the brain's spontaneous activity in deep hibernation. Apparently the activity of the brain in deep hibernation may be thought of as related to two states. *a*) There is activity related to homeostatic mechanisms at low temperatures, which seem to be functioning in a remarkably effective manner. The maintenance of brain temperature within $\pm 0.05^{\circ}$C at a few degrees above environmental temperature during deep hibernation should be emphasized. The brain activity includes, for example, the motor cortical discharge as related to muscle tone (Fig. 16 *A*) and the maintained activity of the septal and hypothalamic areas (as opposed to the relative silence of the lateral geniculate and mesencephalic reticular formation), probably related to maintained autonomic control (Figs. 12 and 14). Note that these records at a lower brain temperature and in sustained deep hibernation appear more like desynchronized records typical of an alert state in a non-hibernating squirrel but merely at a lower amplitude (close to 10 per cent of normal) than records from animals entering hibernation (Fig. 11). *b*) There is activity not obviously related to homeostatic mechanisms, for example the repetitive patterns already mentioned even

in relatively "silent" areas and purely sensory areas (olfactory bulb) quite unrelated to known environmental inputs.

This latter activity may be the activity needed to keep the particular area functioning; perhaps if stopped for too long some dynamic patterning may be lost (perhaps something learned or innate). Or, it may be related to the nervous system being poised at all times for an arousal.

It should be pointed out that the relative quiescence in some areas — the mesencephalic reticular formation, for example (Fig. 16 *B*) — does not imply a temperature depressed excitability, for as soon as the squirrel was touched and the stimulus withdrawn, activity immediately appeared, remained and grew (Fig. 16 *C*).

The pentobarbital experiments should be interesting ones to continue. It makes one wonder whether it is possible in the deeply hibernating mammal to wipe out the brain's spontaneous activity, even transiently, without producing an animal incapable of arousal.

Special properties of the central nervous system of the hibernator. The ability of the squirrel in deep hibernation with brain temperatures close to 6°C, to focus its attention, to attempt to localize stimuli in space, discriminate, vocalize and adjust its posture beside maintaining autonomic regulations is remarkable, particularly when it is realized that there is a 90 per cent reduction in the amplitude of the general electrical activity of the brain. If only 10 per cent of the total neuronal population is active, on the average, then I suspect it is a "carefully selected" 10 per cent rather than a random distribution.

The maintained excitability of the nervous system despite low cerebral temperatures is not unexpected since, of course, it is known that autogenous complete arousals are possible. However, the immediacy, degree and growth of the central response to a simple stimulus despite the low temperature (Fig. 16) may call for temperature-compensated synaptic transmitter mechanisms.

One of the more specialized properties of the hibernating central nervous system is its ability to maintain homeostatic mechanisms so well at low cerebral temperatures. However, it is felt that the most specialized cerebral property lies in the hibernator's ability to turn down neuronal activity to a level, at 6°C brain temperature, which does not interfere with complex behavior, with arousability, with instincts and probably a myriad of learning.

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DISCUSSION FOLLOWING STRUMWASSER'S PAPER

FOLK congratulated the speaker but noted that a correction is essential: the "test drops," he believes, are improbable in the case of the thirteen-lined ground squirrel. In experiments over a 2-year period using a controlled constant environment, it was noted that animals would drop into hibernation immediately without "test drops." On one occasion, six animals entered

hibernation within less than 24 hours after leaving the warm room. STRUMWASSER replied that test drops are not phenomena that can be ascertained by mere visual observation of the hibernating animal; continuous temperature recording of the undisturbed animal in a properly controlled environment is necessary to ascertain whether test drops occur or not. If such experiments are performed with the thirteen-lined ground squirrel and entrance into deep hibernation (core temperature down to 6°C and lower) is observed to occur in one continuous temperature decline, then these animals belong in Group I or II of the scheme outlined in his paper.

LYMAN then substantiated STRUMWASSER'S observations by remarking that he was sure such "test drops" occur with *Citellus beecheyi*. He thought, on the other hand, that it sometimes occurred with the thirteen-lined ground squirrel and the woodchuck, and sometimes not. He stated that sometimes these animals when put in the cold would go into hibernation immediately without a test drop, but at other times they would go "up and down with several test drops."

FOLK commented on the preparatory state for hibernation, pointing out that it, too, must be an internal rhythm. He raised the question of photo-periodicity as the preparatory stimulus for hibernation. STRUMWASSER noted that only precisely controlled environments would give such answers.

GRIFFIN asked whether the brain temperatures given always referred to temperatures taken at the same spot in the brains of experimental animals. STRUMWASSER said the thermocouple was within 3 mm of the same area. GRIFFIN noted that Hammel working on dogs saw gradients in brain temperatures. He asked if gradients appeared in areas of little circulation during hibernation. STRUMWASSER said he would not expect such gradients in brain temperature. GRIFFIN asked what the lowest temperature he recorded was. STRUMWASSER said he never saw a brain temperature below 6°C, and believed this was in some measure related to the California species he was working with. He also noted that it is quite possible that brain temperature is regulated at a considerably higher level in deep hibernation than the rectal temperatures so far reported in various species.

GRIFFIN said he assumed the electrodes used were fairly big. STRUMWASSER replied that small stainless steel wires

37μ and 80μ in diameter, were chosen to minimize damage for subcortical recordings.

BISHOP asked what would happen to the "test drops" if the environmental temperature was lowered very gradually to give adaptation of sense organs. STRUMWASSER said that one would have to do this over about a 15-day period very slowly, which would be a pretty tricky affair, but in his belief, "test drops" would still occur. BISHOP doubted this.

XVI

THE MECHANISMS OF HYPOXIC TOLERANCE IN HIBERNATING AND NON-HIBERNATING MAMMALS[1]

By Robert W. Bullard, George David and C. Thomas Nichols
Department of Physiology
Indiana University School of Medicine
Indianapolis, Indiana

Different mammalian species possess varying degrees of tolerance to hypoxia. Much of the work on comparative altitude physiology is reviewed by Denzer (1950). In general, the poikilotherms are extremely tolerant to almost complete anoxia or altitudes well above 80,000 feet. Likewise the bats, perhaps because of their poikilothermic nature, are extremely tolerant to altitude. Diving homeotherms are also quite tolerant to brief hypoxic periods (Prosser, 1950). Spallanzani first noted in 1803 that bats and marmots had a higher degree of tolerance to oxygen deficiency. Many experiments were done with animals in the hibernation state throughout the nineteenth century. Some were relatively crude such as those of Carlisle (see Biörck *et al.*, 1956), who in 1805 submerged the hibernating hedgehog in water for 30 minutes with no untoward effects. Hiestand *et al.* (1950) have shown that hibernating adult homeotherms are more tolerant than non-hibernating adult homeotherms.

There are many possible ways of explaining the nature of the increased tolerance of the hibernators. For example: (1) the hibernators have been shown to utilize unusual vasomotor control during the dehibernation process (Adolph and Richmond, 1955; Lyman and Chatfield, 1955). Can they call forth this mechanism of restriction of blood flow to only vital regions during a hypoxic exposure as diving mammals can? (2) Hibernators have likewise been shown to be capable of "volitional" body cooling, heart rate decrease and metabolism decrease associated with entrance into hibernation (Lyman, 1958). Does the hibernator upon hypoxic exposure utilize such mechanisms as protective responses? (3) It has been suggested that in hibernation

[1] This project was supported in part by the Research and Development Division, Office of the Surgeon General, Department of the Army under Contract No DA-49-007-MD-947.

anaerobic metabolism is an important share of the total metabolism (Klar, 1951). Can hibernators in the non-hibernating state make greater use of these metabolic pathways than other species? (4) The limited data on oxygen-hemoglobin dissociation curves indicate that the hibernators possess hemoglobins of high oxygen affinities (Prosser, 1950; Barker, 1957). Is this of survival value during hypoxic exposure?

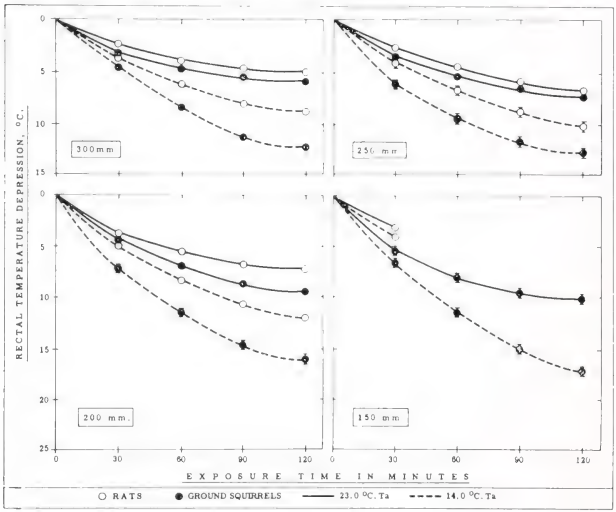

Fig. 1. Changes in rectal temperature of rats and ground squirrels taken to various altitudes at the rate of 4000 feet/min at chamber temperatures of 23°C and 14°C. Standard error of the mean is indicated when six or more animals were used for the determination of a curve.

In this paper some physiological responses to hypoxia are reported for several mammalian species in an attempt to account for differences in hypoxic tolerance.

Body temperature changes upon hypoxic exposure. Figure 1 shows the changes in colonic temperatures of rats (Wistar strain) and thirteen-lined ground squirrels (*Citellus tridecemlineatus*) of similar body weight upon exposures to various degrees of hypoxia in an altitude chamber. The rates of cooling increased as barometric pressure decreased and at all barometric

pressures the ground squirrels always cooled faster than did the rats. The curves tended to level off at a lower body temperature as the exposure progressed. The ground squirrels at 200 mm Hg and 150 mm Hg cooled at approximately the same rate, although there is a marked difference in oxygen deficiency. The ground squirrels at 150 mm Hg and at a chamber temperature of 23°C cooled almost as fast as those at 14°C. If the cooling constant

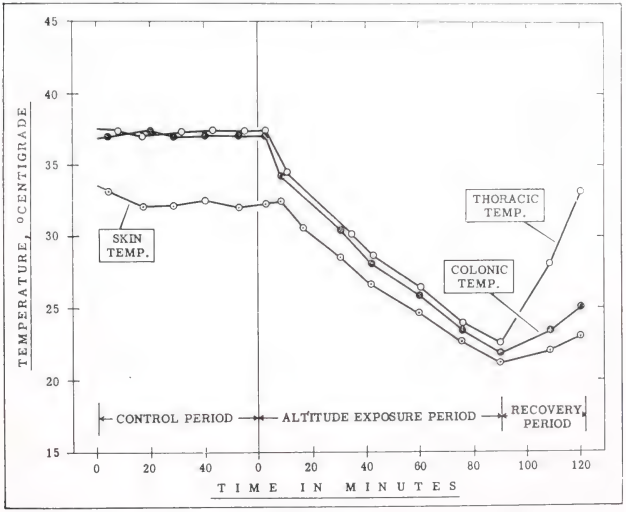

Fig. 2. The changes in body temperature of one squirrel taken to a 150 mm Hg barometric pressure at a chamber temperature of 14°C.

is estimated from Newton's law of cooling, which takes into account the difference between body temperature and ambient temperature, the constant is higher in the squirrel at 23°C. This may be indicative that the cooling process is of a regulated nature and that the squirrel can utilize a maximum cooling procedure if necessary. In another series of experiments, the living ground squirrels in the first 30 minutes of exposure to 150 mm Hg cooled faster than dead rats, and at approximately the same rate as dead ground squirrels.

Localized temperature changes upon hypoxic exposure. Figure 2 shows the curves for various body temperatures obtained for one ground squirrel. Typically, the colonic and

thoracic temperatures fell in a parallel fashion. Only very slight temperature gradients between abdomen and thorax were seen during the hypoxic exposure. At the end of the exposure the typical arousal response is seen with immediate and rapid warming of the thorax followed by a more gradual increase in rectal temperature. The skin temperature shown was obtained from the lower back. In all cases the skin temperature never fell as rapidly as the rectal or thoracic temperatures at the beginning of hypoxic exposure. In some cases it tended to stay constant and in others it rose slightly. After approximately 10 minutes the skin temperature started to fall and the three temperatures approached the same value. A delayed fall in surface temperature coincident to a rapidly falling core temperature can only be explained by vasodilation of peripheral vessels. Thus, in hypoxia, the ground squirrel was not making an attempt to conserve body heat by vasoconstriction. Similar results were obtained with the hamster and rat. Even though the hibernator possesses the machinery of the diving mammal circulatory reflex, it does not utilize this in hypoxia, as has also been reported by Adolph and Richmond (1955). However, at the end of the hypoxic exposure the hibernator immediately utilizes a selective vasoconstriction for rapid rewarming. A lowered body temperature is of little detriment to a hibernator because of this great rewarming ability.

TABLE I

Time of Death at 150 mm Hg in min.

Chamber temperature °C	Rat		Squirrel	
	Time of death	Range	Time of death	Range
38°C	2.8	0 - 6	14.2	9 - 25
33°C	5.0	0 - 7	16.4	6 - 33
28°C	8.0	5 - 10	35.3	18 - 51
23°C	12.7	10 - 19	Survived 2 Hours	
18°C	18.0	13 - 23	" " "	
14°C	19.7	11 - 48	" " "	
14°C (shaved)	27.7			
8°C	48	27 - 68	" " "	
	Survived 2 Hours[1]			

[1] Rats survived if precooled by exposure to 8°C for 1 hour before the hypoxic period.

Temperature depression and survival. Table I indicates the importance of body cooling in hypoxic survival. The rate of body cooling could be altered by simply changing the chamber temperature. The time of "death" was taken as the time respirations ceased. Electrocardiograms were taken but showed great variability with arrhythmic, low voltage waves persisting for some time in many animals. At 38°C the survival times of both the rats and the ground squirrels were severely limited. As chamber temperature decreased, survival times increased. At 23°C the ground squirrels survived the 2-hour exposure period. At a chamber temperature of 8°C, the rats were capable of surviving the 2-hour exposure period. However, in these experiments a 60-minute control period prior to hypoxia was maintained, and during this time, at an ambient temperature of 8°C, considerable cooling of the rat took place which may have enhanced survival. In both species, if the animal could lower its body temperature to below 30°C in the first 30 minutes of exposure to a barometric pressure of 150 mm Hg, it could survive the 2-hour period. Four shaved rats were exposed to hypoxia at 14°C, and showed an increase in survival of 8 minutes when compared to controls. However, the shaved rats did not cool as rapidly as the ground squirrels at this temperature. An important finding here is that at all ambient temperatures, including those at which no body cooling was possible, the hibernator outlived the non-hibernator.

Figure 3 represents a plot of survival times of several species at 150 mm Hg versus various environmental temperatures. It can be seen that the cooler the chamber, the greater is the survival. However, the hamster (*Mesocricetus auratus*) has greater survival time as does the ground squirrel but did not cool as rapidly as the squirrels at a barometric pressure of 150 mm Hg. At a chamber temperature of 23°C the cooling undergone by the hamster was only about half of that undergone by the squirrel. Although body temperature decrease is important in hypoxic survival *it is not the only factor involved.* Hiestand *et al.* (1950) reported that the ground squirrel was more resistant to hypoxia than the hamster. Our data indicate that the hamster has the greater tolerance. However, different experimental approaches as to the rate of ascent and to the final degree of hypoxia were used.

Survival benefits of body temperature depression. It can be demonstrated that body cooling enhances survival in hypoxia. Body cooling in accordance with Van't Hoff's law will decrease

the metabolic oxygen requirement of the animal thus forestalling anoxic damage. Another less obvious benefit is that oxygen volume and tension will be greater in the cooler alveolus due to the diminished vapor tension of water. For example, in the lung of the euthermic animal exposed to a barometric pressure of 150 mm Hg, 47 mm Hg or approximately one-third of the pressure in the lung will be due to water vapor pressure. In

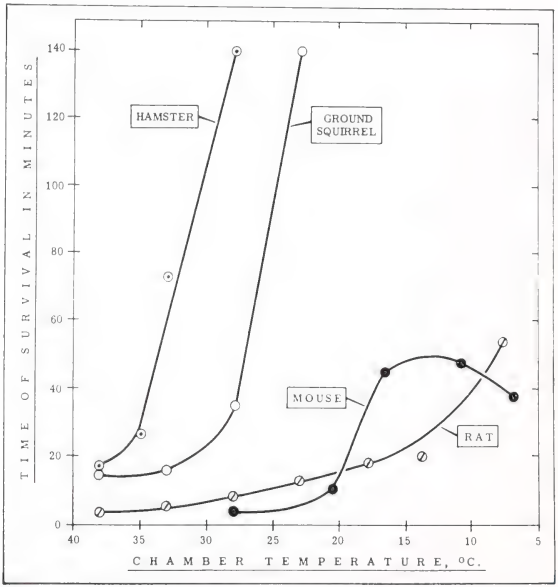

Fig. 3. Survival times at 150 mm Hg barometric pressure and at various chamber temperatures for hamsters, ground squirrels, rats and mice.

the hypothermic animal at 30°C, 31 mm Hg or approximately one-fifth of the lung pressure is due to water vapor pressure. As water vapor pressure is decreased the oxygen tension will be increased. This increase may mean the difference between death and survival.

Studies (Barker, 1957) have shown that a major factor in the tolerance to hypoxia of several mammalian species is the affinity

of hemoglobin for oxygen. Cooling of the blood will increase the affinity of hemoglobin for oxygen or shift the hemoglobin dissociation curve to the left much like that occurring with altitude adaptation. This likewise may account for survival of the cooler mammal.

The mechanism of body temperature depression. What is the nature of this cooling response upon hypoxic exposure? The cooling curves indicate that as exposure continued the temperatures tended toward a new lower level. This may indicate one of the following: (1) that an equilibrium has been established between lowered metabolism and reduced heat loss due to decreased temperature gradients between animal and environment; (2) that regulatory mechanisms in hypoxia are now in action; or (3) that this new temperature level represents a new regulated level permitting survival during the stress of hypoxia.

The peripheral vasodilation which is seen may be interpreted as an attempt at regulation of body temperature at a preferred lower level or hypoxic interference on blood vessels directly or through the vasomotor centers.

These experiments indicate that the cooling is of survival value but they do not indicate whether or not the cooling is simple hypoxic interference upon temperature regulation or an actual regulated response. There is evidence from two Hungarian laboratories that this response is regulated in the rat. Donhoffer *et al.* (1957) have shown that appropriate bilateral epithalamic lesions will abolish the hypoxic cooling response and the hypoxic decrease of oxygen consumption, thus implicating the involvement of the central nervous system. Járai and Lendvay (1958) have exposed rats to 400 mm Hg barometric pressure and followed the oxygen consumption and cooling curves. In rats given injections of dinitrophenol, oxygen consumption and heat production were increased, yet the degree of rectal temperature decrease was the same as that seen in the controls. They interpreted this as an indication that the hypoxic temperature depression is of a regulated nature.

The hibernating mechanism as a factor in hypoxic tolerance. It appears that there are mechanisms other than simple body cooling that *protect the hibernator in hypoxia.* It was seen that the ground squirrel would outlive the non-hibernating rat even though the ambient temperature was such that no body cooling could occur. The hamsters did not cool rapidly at higher temperatures and showed the greatest survival ability. When the tolerances of individual animals were studied, it was

seen that those animals surviving longest at any set of pressure and temperature conditions did not always show the greatest rate of temperature depression. Perhaps hypoxic survival is dependent upon a marked reduction of the metabolic rate. Popovic (personal communication) has demonstrated that the metabolism of a hibernator in natural hibernation is lower than that of the hibernator artificially cooled to the same body temperature. Is it possible that the hibernator not in the hibernating state

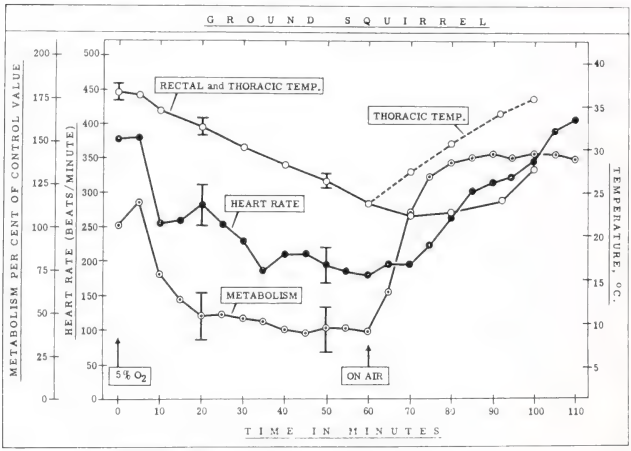

Fig. 4. Changes in metabolism (O_2 consumption), body temperatures and heart rate occurring when ground squirrels breathed a 5-6 per cent mixture of oxygen in nitrogen at a chamber temperature of 12°C. Each curve represents a mean of six experiments. Standard error of the mean is indicated at selected times.

when confronted with hypoxic stress can simulate the same mechanisms utilized when entering hibernation?

The recent data (Lyman, 1958) obtained with chronic preparations on entrance into hibernation have given us criteria which can be used to determine whether or not responses to hypoxia are similar to those of hibernation. In the entrance into hibernation, declines in heart rate and metabolism occur. These decreases are not temperature-dependent as body temperature falls at a slower rate than does either heart rate or metabolism.

Likewise, during the entrance into hibernation peripheral vasodilation may be occurring. As an experimental approach these criteria were tested on animals exposed to cold and hypoxia.

In the first series of experiments, hamsters, rats and ground squirrels were restrained in small cylinders and placed in a 12°C water bath. Electrocardiograms, skin, chest, and rectal temperatures were continuously recorded. Oxygen consumption was measured according to a method described by Adolph (1950). Carbon dioxide production was similarly measured.

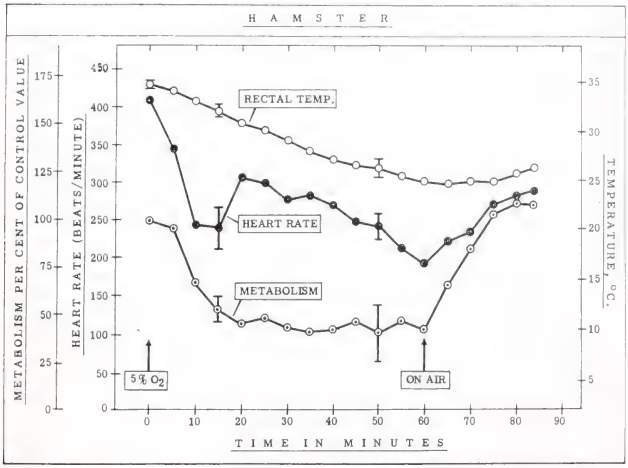

Fig. 5. Changes in metabolism (O_2 consumption), body temperatures and heart rate occurring when hamsters breathed a 5-6 per cent mixture of oxygen in nitrogen at a chamber temperature of 12°C. Each curve represents the mean of six experiments. Standard error is shown at selected times.

Figures 4 and 5 represent the data obtained on six hamsters and six ground squirrels when the air going through the cylinder was changed to a mixture of 5 to 6 per cent oxygen in nitrogen. Upon hypoxic exposure a decrease in heart rate not dependent upon body temperature decrease occurred. For individual animals, the heart rate decrease was not consistent but varied considerably, dropping rapidly at times, rising slightly and

plateauing at other times. After the first large decrease in heart rate, the mean heart rates showed a generally slower decline which was perhaps dependent upon decreasing heart temperature. The rise in heart rate upon the end of the hypoxic exposure appeared to be temperature dependent but with a higher temperature coefficient than that of the latter part of the hypoxic exposure.

The data indicate that there was a decrease in metabolism, as expressed in per cent of the prehypoxic exposure value, not dependent on body temperature decrease. Considerable fluctuation of metabolism was seen in individual animals. Upon the end of the exposure, metabolism rose sharply and did not appear to be body temperature dependent. The metabolism data of these experiments must be considered with extreme caution. The prehypoxic exposure metabolism is not basal, but represents that of a restrained animal exposed to cold. Metabolism was above basal for two reasons: (1) increased thermogenesis due to cold exposure, and (2) occasional struggling of the animal. These experiments must be repeated using unrestrained animals trained to rest quietly in a cylinder.

The responses obtained appear to meet the criteria of hibernation entrance mechanisms, in that there are non-temperature-dependent decreases in heart rate and metabolism and, as has already been shown, peripheral vasodilation. It may be argued quite successfully that all of these responses are hypoxic responses and only similar to hibernation responses by coincidence. The chief objection is that rats, non-hibernators, exposed to 6 per cent oxygen in similar experiments also showed very sharp non-temperature dependent decreases in metabolism and heart rate which were followed by immediate death. At higher oxygen tensions, 75 mm Hg or 10 per cent, which allowed survival, the rat showed more gradual and steady declines in heart rate and metabolism which again were not strictly temperature dependent. In these experiments the rats could not rewarm themselves following the hypoxic exposure, and body temperature, heart rate and metabolism continued to fall at the same rate.

In a series of experiments which is being performed in this laboratory at the present time, heart rates are measured while the animals are taken to altitude at varying rates in the chamber. The results so far may be described briefly as follows. If the chamber temperature were high and decompression slow, an increase in heart rate of rats and ground squirrels would be seen

followed by a rapid decline not dependent on thoracic temperature decline. Hamsters did not show this increase at any temperature but always showed a very rapid decrease in heart rate. Perhaps this is related to the increased tolerance of the hamster. If the chamber were colder and decompression rapid, the squirrels likewise would show a very rapid decline in heart rate which was not temperature dependent. Often the heart rates would climb to higher levels again and then decline in a temperature dependent fashion.

It appears from these recent experiments that cold and rapid onset of hypoxia enhance the heart rate depression in the squirrel. It may be speculated that the cold ground squirrel is partially "set" to enter hibernation, and the hypoxia serves as a trigger. However, we cannot conclude that these hypoxic responses are the same as hibernating responses in the hibernating species. The conclusions of Donhoffer *et al.* (1957) and Járai and Lendvay (1958) are that mammals possess mechanisms for depression of metabolism and temperature in hypoxia. Is it these mechanisms that the hibernators use for entering natural hibernation? With the widespread occurrence of hibernating animals in the different mammalian orders it hardly seems likely that parallel evolution of hibernation mechanisms could have occurred. It is more likely that the hibernators make use of equipment already present in these orders. The evidence for neural regulation of metabolic reduction in rodents has been presented (Donhoffer *et al.*, 1957; Járai and Lendvay, 1958). Perhaps the hypoxia brings about a forced metabolic reduction similar to the "active metabolic reduction" of hibernation as described by Lyman (1958). It may be that hypoxia can initiate the neural activity which is involved in hibernation but not the long-term endocrine regulation.

Hypoxia and artificial hibernation. Differences between hibernation and artificial hypothermia exist. Before we conclude that they are completely different we must critically examine all methods of inducing hypothermia. The responses obtained in cold and hypoxia are similar if not the same as the responses of entrance into hibernation. Is the end result the same? Ground squirrels were exposed to 150 mm Hg barometric pressure in a 7°C chamber for 5-6 hours. During this period all body temperatures fell to about 2 degrees above the chamber temperatures. Heart rates ranged from 7 to 27 beats per minute. When atmospheric pressure was restored to normal, temperatures remained the same. Heart rates remained in the above range and were

extremely arrhythmic as has been reported for true hibernation (Lyman, 1959). After four hours the chamber temperature was increased to 15°C. When the chamber temperature reached approximately 15°C, rapid rewarming of the squirrels began with chest temperatures and heart rates rising rapidly as has been reported for arousal from natural hibernation (Lyman and Chatfield, 1955). Oxygen consumption data have not been obtained, and it is not known whether or not these squirrels will rewarm spontaneously from extremely low body temperatures.

These squirrels may be in a state of "neural" hibernation but not "endocrine" hibernation. We hope that this symposium will establish definitive criteria for hibernation so that such questions may be answered.

Conditioning of responses to hypoxia. It has been noted that after ground squirrels were used for several hypoxic exposures, for example, three exposures in eight days, responses to hypoxia changed. During the control prehypoxic period these squirrels maintained a constant thoracic temperature while rectal temperatures fell rapidly. Immediately upon the onset of decompression of the chamber, before a real hypoxic environment had been established, thoracic temperature fell rapidly toward the level of the rectal temperature. When the thoracic temperature decreased to less than a degree above the rectal temperature, both temperatures fell in a parallel fashion. Heart rate in these animals declined rapidly and did not show the fluctuations found in squirrels upon the first exposure. Metabolism also fell rapidly without fluctuation. At the end of the hypoxic exposures rewarming was faster, heart rate increased faster and metabolism increased faster and to a much higher level than those of first-run squirrels. In a limited series of rat experiments the responses to hypoxia did not show any statistically significant changes with repeated runs.

The change in response of the squirrel with repeated exposures may represent adaptations at cellular levels or a conditioning at the regulative levels. If much central nervous system involvement is necessary for these responses then the development of a conditioned reflex is a strong possibility. The fact that the thoracic temperature began to decrease before a real hypoxic environment had been established indicates that it may be a central nervous system response. More experiments are needed to truly define the nature of these changes.

Discussion

It has been shown that rapid body cooling enhances survival in hypoxic exposures. It has likewise been pointed out that cooling in itself will not account for species differences in hypoxic resistance. None of the mammals studied utilized the vascular responses similar to those utilized by diving mammals. There is evidence that the hypoxic metabolic depression and temperature depression are regulated by the central nervous system and are not entirely direct effects of hypoxia on tissues. It can be stated that hypoxic responses and hibernating responses appear quite similar; whether or not they are the same responses remains to be conclusively proven. Perhaps when more data are obtained for both hibernators and non-hibernators for exactly the same type of hypoxic exposure the answer will be found. It may be speculated that the forced metabolic depression in hypoxia is of benefit to the animal.

Other possibilities must still be considered. One factor which may give the hibernator enhanced survival in altitude is the possession of hemoglobin of high oxygen affinity. The very limited published data indicated that this may be the case. For example, the marmot hemoglobin has a one-half saturation oxygen tension of 23.8 mm Hg (Prosser, 1950), the hamster, approximately 20 mm Hg (Barker, 1957), the rabbit 32 mm Hg, mouse 72 mm Hg, and rat 40 mm Hg (Prosser, 1950). These values should be considered with caution as the "physiological" curve may be different from the laboratory derived curve. The hemoglobins of the hibernators seem to have high oxygen affinity. It has been demonstrated that the higher the oxygen affinity the greater the hypoxic tolerance (Barker, 1957). More data is needed for the rest of the hibernating species before this factor can be evaluated.

It has been pointed out that anaerobic metabolism may be the predominant metabolism of hibernation (Klar, 1951; Björck *et al.*, 1956). However, further biochemical studies are needed, especially during the hypoxic exposure. Whether or not the hibernator may utilize anaerobic pathways more extensively in hypoxia is another possibility that must be investigated.

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DISCUSSION FOLLOWING BULLARD'S PAPER

DAWSON inquired as to the change in RQ during the metabolic changes of hypoxia. BULLARD replied that he measured not RQ but respiratory exchange ratio which is changed by ventilation, but that the values are not as low as reported for hibernation since the RQ for hibernation is a reflection essentially of a prolonged hypothermia in which body fat is utilized.

JOHANSSON pointed out that BULLARD used stoppage of respiration as the termination point of biological activity following hypoxia, but he wondered about the heart activity which continued. BULLARD said they had not seen ventricular fibrillation, but only low voltage waves which continued for some time. This time was highly variable; respiratory cessation was also variable but gave a better end-point.

JOHANSSON stated that many believe that the basic difference between hibernators and non-hibernators is the ability of the autonomic nervous system to be functional at a low temperature. Of even greater importance, JOHANSSON felt, is a basic difference in metabolic patterns between the two kinds of animals. One way of showing this is to remove the heart and perfuse it with Tyrode solution. In this way nervous and humoral influences would be removed more or less completely. In such an experiment, the heart of a hibernator will stop beating at a much lower temperature than a non-hibernator's heart.

ADOLPH volunteered a method for clarifying the distinctions between hypoxia and hypothermia. In order to avoid hypothermia, if one wishes to study hypoxia alone, thermistors are placed in the animals, which operate a heating device. In such a situation, the hypoxic animal continuously activates the instrument to keep itself warm, because the animal will inevitably tend to cool in hypoxia. ADOLPH also pointed out that in hypoxia there are diminutions in oxygen consumption, and often in heart rate, although the hypoxic animal continues to breathe excessively in the presence of low oxygen.

BULLARD stated that Hungarian papers are available which describe an abolition of the depression in body temperature and in metabolism following hypoxia by appropriately placed brain lesions.

XVII

ON THE CARDIAC RESPONSE IN HIBERNATION AND INDUCED HYPOTHERMIA

FUNCTIONAL, PATHOLOGIC AND METABOLIC ASPECTS[1]

By H. S. S. Sarajas
Institute of Physiology, University of Helsinki
Helsinki, Finland
and Wenner-Gren Cardiovascular Research Laboratory
Stockholm, Sweden

In the course of the last few years it has become increasingly evident that natural hibernation and induced hypothermia in non-hibernating mammals represent fundamentally different physiologic states. In natural hibernation the main vital functions continue, even if profoundly retarded, down to body temperatures near zero, a considerable degree of homeostasis being maintained. That the neuroendocrine system, for example, retains a relative integrity in deep hibernation is suggested by the mere fact that the hibernating animals are capable of arousing themselves by various external stimuli, even including intense cold. Non-hibernating mammals rendered hypothermic by the customary technic, consisting of a suppression of the thermoregulatory defense by anesthetics followed by intense cooling of the body, behave in a different fashion. With decreasing body temperature their vital functions are also retarded. However, at body temperature levels of about 10° to 20°C fatal cardiac crises usually supervene even when artificial respiration is employed to maintain sufficient blood oxygenation. Moreover, mammals in deep tolerable hypothermia are generally unable to arouse themselves spontaneously no matter what the external stimulus. The only means of reviving them is by the external or internal application of heat. Then, when body temperatures above the levels of the so-called "cold narcosis" are attained, and provided there is no residual anesthesia, the hypothermic mammal may eventually rewarm itself by means of its thermoregulatory mechanisms.

[1] Support for the writing of this survey and for some of the experiments summarized here was provided by the Emil Aaltonen Foundation and the Sigrid Juselius Foundation.

The main physiologic differences between hibernation and induced hypothermia have been authoritatively discussed by Popovic (1952), Kayser (1955), and Lyman and Chatfield (1956), among others. The present paper is an attempt to analyze the cardiac response in hibernation and induced hypothermia with reference to some differences in the cardiac function, cardiac pathology and myocardial metabolism. The cardiac reactions in hibernation will be contrasted with those in hypothermia, induced in anesthetized mammals under artificial respiration by surface cooling. As is known, hypothermia of this type has been most widely studied by physiologists and most widely practiced in surgical therapy. Moreover, such a restriction seems justified in view of the fact that, depending on experimental variables, a wide variety of physiologic states may occur in hypothermia, just as they may in normothermia.

Cardiac function. Generalizing, it may be said that with decreasing body temperature the heart function is progressively retarded in both hibernation and induced hypothermia. However, closer observations on the heart rate, cardiac rhythm and configuration of the electrocardiographic records in association with hibernation and hypothermia reveal remarkable differences.

At first, the definitely different critical body temperatures for the heart function may be recalled. In hibernating animals a coordinated heart function is maintained down to body temperatures near zero (Suomalainen and Sarajas, 1951; Sarajas, 1954; Björck and Johansson, 1955; Dawe and Morrison, 1955; Lyman and Chatfield, 1956). According to Adolph (1951), infant non-hibernating mammals with insufficient thermoregulation can tolerate body temperatures of the same general order. Adult non-hibernating mammals, on the contrary, generally succumb with ventricular fibrillation or cardiac standstill when body temperature levels ranging from about 10° to 20°C are attained. This terminal cardiac crisis is usually preceded by ventricular ectopic beats and nodal or idioventricular rhythms (Crismon, 1944; Bigelow *et al.*, 1950; Björck and Johansson, 1955). Finally, it may be mentioned that isolated heart preparations from hibernating and adult non-hibernating mammals stop functioning at temperature levels closely corresponding to the critical body temperatures for heart function in these two groups of mammals (Hirvonen, 1956).

Until recently, information on the earliest cardiac events in animals entering hibernation has been scanty. However, Lyman (1958) has shown that in woodchucks the heart rate tends to

decline prior to the fall in body temperature, and judging from the subsequent report of Strumwasser (1959a) the same holds for the California ground squirrel. These observations are also compatible with our observations on hedgehogs (Suomalainen and Sarajas, 1951; Sarajas, 1954). All the investigators agree that during entrance into hibernation there is initially a steady decline in the heart rate fairly proportionate to the fall in body temperature (Suomalainen and Sarajas, 1951; Sarajas, 1954; Dawe and Morrison, 1955; Lyman, 1958; Strumwasser, 1959a). In deep hibernation, however, the heart rate appears largely independent of the body temperature levels; prolonged periods of extreme bradycardia are interrupted by short periods of relative tachycardia. This phenomenon has been shown in different hibernators including the hamster (Chatfield and Lyman, 1950; Lyman, 1951), the hedgehog (Suomalainen and Sarajas, 1951; Sarajas, 1954; Biörek and Johansson, 1955; Dawe and Morrisson, 1955), the four species of ground squirrel (Lyman, 1951; Dawe and Morrison, 1955; Nardone, 1955; Kayser, 1957; Landau and Dawe, 1958; Strumwasser, 1959b), and the woodchuck (Lyman, 1958). In the framework of the present discussion this phenomenon is of significance in that it clearly demonstrates that even in deep hibernation the heart function is influenced by neural and/or endocrine mechanisms.

The cardiac reactions in hibernating mammals roughly characterized above differ essentially from those in non-hibernating mammals subjected to induced hypothermia. The changes in the heart rate in the early stages of hypothermia appear to be largely determined by the depth of anesthesia. In lightly anesthetized animals there occurs an increase in the heart rate in the early cooling period, following which, at about 35°C, the heart rate begins to fall in a fairly linear fashion (Grosse-Brockhoff and Schoedel, 1943a; Prec *et al.*, 1949; Bigelow *et al.*, 1950; Biörek and Johansson, 1955). This initial increase in the heart rate is obviously effected by direct cutaneo-cardiac reflexes, increased "venous return" on shivering, and generalized sympathetic activation elicited by cold stimulus, and is even reinforced by the increased excitability of the medullary (Grosse-Brockhoff and Schoedel, 1943b) and hypothalamic (Koella *et al.*, 1954) centers reported to occur in the early stages of hypothermia. When deeper anesthesia is used, this initial activation of the heart function on cooling is largely abolished; then the heart rate tends to decline linearly from the very beginning of cooling (Hook and Stormont, 1941; Bigelow *et al.*, 1950; Biörek and

Johansson, 1955). Nevertheless, it may be pointed out that the progressive bradycardia by induced hypothermia is somewhat relative, contrary to that in hibernation. In our experience with dogs, and judging from the data given by others (Hook and Stormont, 1941; Grosse-Brockhoff and Schoedel, 1943a; Bigelow *et al.*, 1950; Biörck and Johansson, 1955), the heart rates characteristic of normal, resting, unapprehensive dogs (Murphy, 1942) are not attained before body temperature levels of about 25°C. This might be largely due to the fact that, for example, pentobarbital and ether, both commonly employed to assist the induction of hypothermia, do considerably accelerate the heart rate, the former apparently by its vagal blocking action (Nash *et al.*, 1956) and the latter by generalized sympathetic activation (Brewster *et al.*, 1953). With these statements in mind, it seems evident that in the early stages of hypothermia there is, at all events, some trend to sympathetic stimulation with a resultant acceleration of the heart rate. Remarkably enough, just the reverse apparently holds for animals entering hibernation, for Strumwasser (1959a) has recently presented evidence that during entrance into hibernation there is a shift of balance toward the parasympathetic nervous system. This would offer a reliable explanation of why, in the hibernating animals, the heart rate begins to decline even before the fall in body temperature.

Several investigators (Grosse-Brockhoff and Schoedel, 1943a; Haterius and Maison, 1948) have found that neither sectioning of the vagi nor atropinization have any appreciable influence on the bradycardia of induced hypothermia. Moreover, the reactivity of the autonomic centers has been reported to be decreased in deep hypothermia (Koella *et al.*, 1954). It appears, then, that the hypothermic bradycardia is mainly effected by the direct depressant action of cold on the cardiac pacemaker activity, and that, on the other hand, deep hypothermia, in contrast to hibernation, results in a breakdown of the homeostatic mechanisms normally controlling the heart function.

The general configuration of the electrocardiograms taken during hibernation and induced hypothermia also reveals definite differences. According to our own observations (Sarajas, 1954), and to those of Biörck and Johansson (1955), and of Dawe and Morrison (1955), the electrocardiographic intervals are fairly uniformly prolonged in hibernating animals, while the configuration of the deflections does not otherwise show any significant deviation from normal. This is in striking contrast to the

electrocardiographic changes accompanying induced hypothermia. In hypothermic animals, the Q-T interval is characteristically more prolonged than the other intervals. Moreover, there are associated changes in the configuration of the deflections. The bizarre deformation of the whole ventricular complex constitutes a particularly prominent feature (Grosse-Brockhoff and Schoedel, 1943a; Lange *et al.*, 1949; Bigelow *et al.*, 1950; Hegnauer *et al.*, 1950; Biörck and Johansson, 1955).

Judging from the electrocardiographic records, and as may well be expected, the functional cardiac changes in hibernation are completely reversible (Sarajas, 1954). According to Chatfield and Lyman (1950), and Dawe and Morrison (1955), the heart rate begins to increase before any increase in the body temperature becomes apparent. It has also been found that with increasing body temperature the heart rate increases, at first slowly and then more rapidly (Chatfield and Lyman, 1950; Suomalainen and Sarajas, 1951; Sarajas, 1954; Biörck and Johansson, 1955; Dawe and Morrison, 1955; Landau and Dawe, 1958). From their pharmacologic observations, Chatfield and Lyman (1950) concluded that the rapid increase in the heart rate during arousal is effected by a mass activation of the sympathetico-adrenal system, as previously suggested by Britton (1928) and Barcroft (1934). Our own observation that in hedgehogs the heart beats at supernormal rates in the later stages of arousal, and that normal heart rates are attained several hours after the body temperature has become normalized (Sarajas, 1954), also seems consistent with this concept. Finally, it may be stated that, at least in hedgehogs, the electrocardiogram following arousal appears completely normal (Sarajas, 1954).

There is somewhat controversial evidence as to the degree of restitution of the cardiac function on rewarming from tolerable levels of induced hypothermia. Fatalities, indeed, are known to have occurred during and following rewarming (Haterius and Maison, 1948; Prec *et al.*, 1949; Fedor *et al.*, 1958). Moreover, Swan (1956) has recently presented evidence of acute circulatory collapse with associated tachycardia in dogs following rewarming from moderate hypothermia. He was emphatic in stating that an animal cooled and rewarmed is not returned to physiologic normality. On the other hand, some investigators maintain that the electrocardiogram is normalized upon rewarming (Hook and Stormont, 1941; Prec *et al.*, 1949; Hegnauer *et al.*, 1951; Santos and Kittle, 1958), while others have recorded residual changes especially in the ventricular complexes (Bigelow *et al.*,

1950; Gunton *et al.*, 1956; Fedor *et al.*, 1958; Helbig and Heinrich, 1958).

Cardiac pathology. In the previous section the evident type difference between the electrocardiographic changes accompanying hibernation and those in induced hypothermia was stressed. Toward the end of the last decade Lange *et al.* (1949) found that artificial respiration even with pure oxygen, as commonly employed in actual experimentation with hypothermia, does not prevent or alter the electrocardiographic abnormalities, including the bizarre deformation of the ventricular complex associated with tolerable hypothermia. Remarkably enough, however, they noted that acidification of the blood, which tends to counteract the shift of the hemoglobin dissociation curve to the left by cold, resulted in a normalization of the electrocardiogram. The same could also be effected by increasing the amount of oxygen physically dissolved in the plasma. As a general result these investigators concluded that in induced hypothermia there may exist cardiac hypoxia, without concurrent hypoxemia. They further suggested that the deaths from acute exposures to cold may be due to hypoxic damage to the heart, of too early a stage to be detected by the routine histopathologic technics.

The theory of cardiac hypoxia in conditions of induced hypothermia was initially advocated by some workers (Bigelow *et al.*, 1950; Hegnauer *et al.*, 1950), but subsequently it has been mainly abandoned (Penrod, 1951; Hegnauer, 1954; Berne, 1954; Jude *et al.*, 1957). Moreover, until recently it has been generally accepted that hypothermia is a safe and non-injurious procedure. There follows some data on our recent studies intended to elucidate the pathology of the heart in association with hypothermia in dogs. As will be seen, the results furnish evidence that in hypothermia the heart may actually suffer from hypoxia, with resultant injury to the myocardium. The dogs, totalling 100 individuals, were subjected to graded hypothermia with or without subsequent rewarming, by use of routine technics. The results, some of which have been reported elsewhere (Sarajas and Nilsson, 1954; Sarajas, 1956), may be outlined as follows: in dogs cooled until fatal termination or held from one to four hours at body temperature levels of about 22° or 27°C without subsequent rewarming, the myocardial wall exhibited slight but detectable fatty degeneration and early necrobiotic changes in the form of coagulative necrosis. In addition, some histochemical evidence of glycogen and potassium loss from the muscle fibers was observed. In dogs subjected to hypothermia of the same

degree and equal duration and then rewarmed and killed 3 days to 3 years following rewarming, definite, even grossly detectable myocardial necroses were found which showed different stages of development and healing according to the length of survival. In both the acute and long-term experiments the lesions were found in the great majority of cases. The dynamics of the lesions corresponded to that of experimental myocardial infarction in dogs which indicates, among other things, that they were pathogenetically related to the hypothermic period. On the other hand, the lesions were mainly restricted to the subendocardial muscle layers of the left ventricle. This furnishes evidence for the hypoxic origin of the lesions, for due to certain peculiarities of the coronary circulatory dynamics the subendocardial muscle layers of the left ventricle are the first to suffer from any type of hypoxia (Raab, 1956; Schütz, 1958). The histochemically demonstrable depletion of myocardial glycogen associated with loss of the intracellular potassium gives further support to the hypoxic origin of the lesions, for hypoxic myocardium is known to lose both glycogen and potassium (Dennis and Moore, 1938; Merrick and Meyer, 1954; Raab, 1956).

For the present not much is known about the mechanisms which would render the heart muscle hypoxic in induced hypothermia. Lowered arterial pressure (Bigelow *et al.*, 1950) in the presence of increased blood viscosity (Hegnauer *et al.*, 1950) with eventual intravascular aggregation of the red cells (Bigelow *et al.*, 1950; Konrad and Zindler, 1958), all known to occur in hypothermia, may be proposed as possible factors. On the other hand, when the tachycardia inherent to the early stages of cooling was discussed, it was concluded that in the early stages of cooling there is at all events some trend to sympathetic activation. This is in harmony with our recent studies (Sarajas *et al.*, 1958), suggesting that the induction of hypothermia generally evokes a stress response, even if some stress manifestations may be obscured by certain specific effects of hypothermia. It may therefore be of interest that, according to Raab (1956), epinephrine, the release of which is inherent to any stress situations, is an hypoxiating agent and is capable of causing cardiac lesions similar to those encountered in the present dog cases. Moreover, Selye (1957, 1958) has recently produced similar cardiac lesions in specially conditioned animals by various stressors including administration of epinephrine, neuromuscular exertion, and cold and hot baths. Also, the shock-like state of rewarming, which was previously referred to, may seriously impair the myocardial

nutrition and thus contribute to the genesis of the cardiac lesions under consideration. Even if, accordingly, the causation of these hypothermic cardiac lesions can only be speculated upon, the fact remains that similar cardiac lesions have recently been found also by other investigators (Cecconi and Parentela, 1957; Fisher *et al.*, 1957; Heinrich and Schautz, 1958). Furthermore, quite recently Hannon and Covino (1958) concluded from metabolic studies of heart slices and homogenates that a mild cardiac hypoxia may exist in hypothermia. The possible functional significance of these cardiac lesions which appear to result from hypothermia has been discussed elsewhere (Sarajas *et al.*, 1956). Suffice it to say that they constitute a plausible explanation for the grave, and even fatal, disturbances in cardiac function associated with induced hypothermia. To our knowledge, there are no reports that pathologic cardiac changes would result from natural hibernation. Neither can such a possibility be seriously considered.

Myocardial metabolism. Generalizing, it may be said that any morphologically demonstrable vital reactions are preceded by a metabolic interference in the tissue involved. It may therefore be of interest that the hypothermic heart, contrary to the hibernating one, appears to suffer from metabolic disturbances. The histochemical evidence for loss of glycogen and potassium from the heart in conditions of hypothermia, as previously mentioned, has been confirmed by chemical methods (Szekeres *et al.*, 1954; Covino and Hegnauer, 1955; Gollan *et al.*, 1957). According to Gollan *et al.* (1957), hypothermia gives rise to a marked potassium loss from the myocardium, which is only slightly aggravated when the hypothermic animal is subjected to acute respiratory hypoxia. Szekeres *et al.* (1954), in turn, have found that hypothermia not only results in a depletion of the cardiac glycogen but also in a loss of the high energy phosphates, both commensurate with those evoked by respiratory hypoxia in normothermic animals. These changes may be of significance at least in two respects. First, they give further support to the concept of cardiac hypoxia in conditions of hypothermia. Secondly, they suggest that vital functions of the cell membrane such as selective permeability and active ion transport, which also are prerequisites for normal cardiac function, are disturbed in the hypothermic heart apparently as a result of failure in the resynthesis of high energy phosphates. In principle, just the reverse cardiac metabolic alterations are encountered in the hibernating animals. According to the chemical and histochemical studies of Lyman

and Leduc (1953) and Zimny and associates (1957, 1958), the glycogen content of the heart is increased in hibernation. Judging from our own determinations of the specific activity of the heart glycogen in awake and hibernating hedgehogs given C^{14}-labeled glucose, this glycogen accumulation in the heart is rather tremendous (Forssberg and Sarajas, 1955). On the other hand, Suomalainen (personal communication) has presented histochemical evidence that the glycogen accumulation in the heart is accompanied by an increase in the intracellular potassium, and the recent studies of Zimny and Gregory (1958) indicate that the cardiac high energy phosphates are maintained during hibernation.

Several investigators including Lyman and Chatfield (1956) and Zimny and Gregory (1958) have proposed that in hibernation the heart is storing glycogen for energy in awakening. As was mentioned, the process of arousal is essentially a mass activation of the sympathetico-adrenal system, which then accelerates heart function and metabolic activity. On the other hand, Landau and Dawe (1958) have concluded from the dark color of the blood and of the mucous membranes of the mouth during arousal that the metabolic activity increases more rapidly than oxygen is supplied. From these statements, and considering the previously mentioned hypoxiating effect of epinephrine, it becomes apparent that during arousal the heart is beating under impending hypoxia. Nevertheless, in this critical period glycolysis apparently safeguards the maintenance of the cardiac energy metabolism, for Zimny and Gregory (1958) have presented evidence that the glycolytic activity in the heart during early arousal is essential in resynthesizing the high energy phosphates. This, in turn, agrees with the generally accepted concept that glycogen in the heart muscle is a reserve fuel to be utilized for the resynthesis of the high energy phosphates under anaerobic conditions only (Raab, 1956). Thus the known tolerance of experimental anoxia by the hibernating animals (Björck *et al.*, 1956) may also be related to the high levels of cardiac glycogen.

From the foregoing it may be concluded that while the hibernating heart is metabolically prepared to sustain the work load imposed by the process of arousal, the hypothermic heart, when faced with the hazardous stage of rewarming, is obviously suffering from metabolic failure.

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DISCUSSION FOLLOWING SARAJAS' PAPER

MENAKER asked if young, non-hibernating mammals which have not yet acquired the ability to thermoregulate showed heart damage on cold exposure. SARAJAS said he could not give any definite answer to the question, yet he believed that young, non-hibernating mammals may tolerate low body temperatures better than adults because they have, e.g., a certain reserve for anaerobic metabolic activity and because their neuroendocrine apparatus only weakly responds to cold.

WIMSATT cited work of C. F. Bond and P. W. Gilbert (Anat. Rec., **125**:646, 1956) which demonstrated a profound slowing of cardiac activity in diving birds when they submerge and during the period of submergence, in contrast to non-diving birds in which the opposite effect is seen. He generalized further that as far as he knew, there were no metabolic rates of specific organs which correlated ideally with a general reduction in metabolism.

XVIII

CIRCULATORY CHANGES IN THE THIRTEEN-LINED GROUND SQUIRREL DURING THE HIBERNATING CYCLE[1]

By Charles P. Lyman and Regina C. O'Brien
Department of Anatomy, Harvard Medical School, Boston
and Museum of Comparative Zoology, Harvard University
Cambridge, Massachusetts

In the past few years the function of the heart in hibernation has received considerable attention. For example, it has been shown that there is a marked decline in heart rate before a decline in core temperature when the animal starts to enter the hibernating state (Lyman, 1958). In hibernation, the heart rate may vary greatly with no apparent changes in body temperature (Dawe and Morrison, 1955; Lyman, 1958). On arousal from the hibernating state the heart rate increases prior to a change in body temperature (Lyman and Chatfield, 1950, and references above). These observations suggest that there must be important changes in the circulation during the hibernating cycle and that measurements of these changes might give further insight into the phenomenon of hibernation.

Although some measurements have been made on the blood pressure of mammals waking from hibernation (Dubois, 1896; Chatfield and Lyman, 1950; Chao and Yeh, 1951) nothing has been reported on the blood pressure of mammals either entering the hibernating state or in natural, deep hibernation. The technique developed by Still and Whitcomb (1956) for chronically intubating the aorta of small mammals gave the opportunity of measuring the blood pressure of hibernators over long periods of time. Measurements could be made as the animal passed from the active condition into hibernation, as it remained in hibernation, and as it aroused from the hibernating state. The tube also offered a means of introducing drugs of known pharmacological effect into the circulation at any point during the hibernating cycle without disturbing the animal. Using in-dwelling thermocouples and electrodes, the body temperature and the electrocardiogram (EKG) could be monitored concurrently with the blood pressure.

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This paper is a description of the changes which take place in the blood pressure, body temperature and EKG during the hibernating cycle of the thirteen-lined ground squirrel (*Citellus tridecemlineatus*), and the effect of drugs during various parts of this cycle.

Materials and Methods

A total of 47 ground squirrels were intubated for this study. Of these, five yielded satisfactory records of the various phases of the normal hibernating cycle and seven others were used successfully in the study of the effect of drugs on the circulation during hibernation. Of the former, continuous records of 2 to 8 days were obtained from single animals. The animals were kept in individual cages in a cold room which was maintained at 6 ±4°C. They were given shavings for bedding and Purina laboratory chow and water *ad libitum*. Animals which had hibernated over protracted periods of time were used preferentially. Prior to intubation, the animals were aroused in a warm room, and then anesthetized with an intraperitoneal injection of pentobarbital sodium (80 mg/kg). The aorta was exposed by an abdominal incision. A small slit was made in the vessel about 1 cm posterior to the renal arteries and a thin polyethylene tube (PE 10, ID .28 mm, OD 0.6 mm), bevelled at the end, was inserted into the slit and pushed rostrally 1.5 cm. No modifications were made in the technique described by Still and Whitcomb (1956) except that the instrument used to make the slit in the aorta was a curved Bard Parker blade (size 12), ground to 0.5 mm width and sharpened to a needle-like point. Once the tube was fastened in place with a tie to the muscles of the back it was filled with heparin-saline (25 mg heparin/10 ml physiological saline) and closed at the distal end with a knot. The abdominal incision was closed and the tube anchored again with a tie at the caudal edge of the incision. The tube was then led subcutaneously to an exit between the scapulae, and fastened to the skin of the back. The animal was given 40,000 units of procaine penicillin and returned to its cage in the cold room.

If intubated animals were observed to re-enter hibernation, they were removed in the hibernating state and fitted with one or two thermocouples made of 34 ga. iron and constantan wire individually protected with PE 10 polyethylene tubing. Usually one thermocouple was fastened subcutaneously in the region near the heart and the other fastened intraperitoneally at the mid-abdomen. The thermocouple wires were led subcutaneously to

the exit between the scapulae. Three silver wire electrodes were sewed into the skin of the back. The tube from the aorta was spliced using a section of #27 hypodermic needle and a long piece of PE 10 tubing. All wires and the tube were passed through a helical spring for protection, and the spring was sewed to the back where the tube and thermocouple wires made their exit from the animal.

The ground squirrel was placed in a round battery jar measuring 23 cm in diameter with food and water and the bedding from its cage. The helical spring was led through a wire screen which closed the top of the jar and was suspended with an elastic band so that the animal could move freely in the cage without being bothered by the cable.

Throughout the chronic experiments, temperatures from heart and abdomen were each recorded every thirty-two seconds on a Leeds and Northrup Speedomax thermoelectric recorder with an accuracy of $\pm 0.25^{\circ}$C. The EKG and blood pressure were obtained every four minutes, for a period of one minute.

Blood pressure was measured directly from the polyethylene tube using a Statham P23D pressure transducer.[2] This was amplified with a Grass low-level DC preamplifier, model 5P1A, and Polygraph DC driver amplifier, model 5.[3] Various sensitivity settings were used during the experiments and a drift of as much as 25 mm Hg. could take place in a twenty-four hour period. However, the machine was calibrated at least twice a day, and more often when exact measurements were required. Thus the accuracy did not vary more than $\pm$ 5 mm Hg which is a slight change compared to those which actually took place in the blood pressure. In order to prevent clotting in the tube, a flow of heparin-saline (0.6 mg/ml) of approximately 0.5 ml per day was perfused through the tube by means of a slowly driven screw-drive syringe. Because it was possible that the length of the polyethylene tube might seriously affect the recorded pulse pressure, various lengths of tubing were tried under known conditions of blood pressure. It was found that, within the conditions of the experiment, neither the varying lengths nor temperatures of the tubes made any appreciable differences in the blood pressure measurements.

The apparatus was kept running day and night during the measurements. At various times, records were obtained of animals in the active condition in the cold, during the process of

[2] Statham Instruments Inc., Los Angeles, Calif.

[3] Grass Instrument Co., Quincy, Mass.

entering hibernation, in the hibernating state, and arousing from hibernation. In the experiments using drugs, the agent was introduced into the animal via the polyethylene tube. In all cases, the approximate dose was determined by giving graded doses of the drug in question to intubated, nembutalized rats. Awake, intubated ground squirrels were given doses below those which affected the rats, and the doses were increased until some effect was noted. Subsequently, a comparable dose was used initially in each experiment and increased gradually if no effect was noted. Periodically, tests were made to be sure that the same amount of heparin-saline solution did not produce a similar result. In order to produce vasodilation individual doses of acetylcholine chloride (Merck[4]) varying from 0.15 to 1.2 mg/kg were given while Benodaine hydrochloride Merck[5] was given at 8 to 41 mg/kg. To produce vasoconstriction l-Norepinephrine (Levophed bitartrate, 0.2%, Winthrop) was used in concentrations of 6 to 44 μg/kg.

Results

The normal non-hibernating ground squirrel in the cold maintained a fairly steady heart temperature of 37 ±1°C. The abdominal temperature averaged 0.5 to 1°C below the heart temperature. Blood pressure and heart rate varied considerably, depending chiefly on the activity of the animal. Often the heart rate was reduced as much as one-half in a few seconds, accompanied by a reduced blood pressure and an increase in pulse pressure. Though this occurred invariably if the animal were alarmed, it also took place for no apparent reason. Over periods when two animals failed to hibernate for several days the mean blood pressures averaged 119 mm Hg, but the highest mean pressure was 158 and the lowest was 76. Highest systolic and lowest diastolic pressures were about 20 mm Hg above and below these figures. Heart rates from the same observations averaged 299 beats per minute, with a high of 468 and a low of 184.

Entrance into hibernation was usually preceded by some sort of activity, for the blood pressure and heart rate rose transiently. After this period of activity there was a sudden drop in heart rate, accompanied by a decrease in systolic and diastolic pressure (Fig. 1). Although the heart rate might decrease to one-third

[4] We are extremely obliged to Merck & Co. of Rahway, New Jersey, for giving us the acetylcholine.

[5] 2-(1-Piperidylmethyl)-1, 4-benzodioxan hydrochloride.

of its original value in fifteen minutes and the blood pressure drop precipitously, still the latter remained in the lower part of the range found in the resting, awake animal.

After heart rate and blood pressure declined, the body temperature started to decrease. Often after a few minutes the heart rate again speeded and the blood pressure rose. This was followed by a rise in body temperature. A second decline in heart rate and blood pressure was again followed by a drop in body temperature. Although the heart and abdominal temperatures were not the same at the beginning of the hibernating state, they

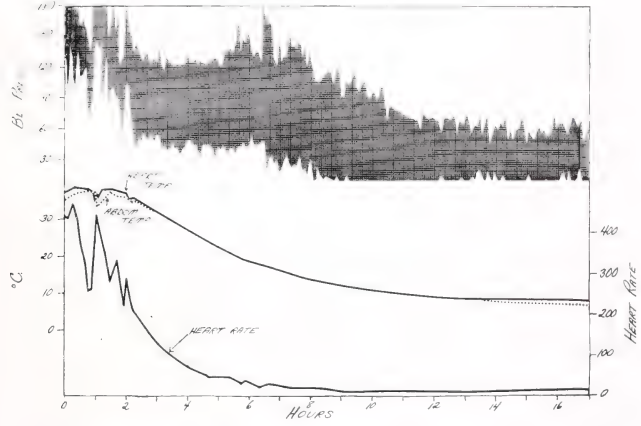

Fig. 1. Blood pressure, heart and abdominal temperature, and heart rate of ground squirrel entering hibernation. Blood pressure in dark area is highest systole and lowest diastole recorded every four minutes for a one-minute period. Note declines in heart rate and blood pressure, followed by body temperature.

soon became identical and remained the same until the animal was near the temperature of the environment. At this time the heart temperature was about 0.5°C above the abdominal temperature and remained so while the animal stayed in hibernation.

During the first part of entrance into hibernation, heart rate and blood pressure were irregular. Bradycardia often occurred for a few seconds followed by tachycardia, with a concurrent decline and rise in blood pressure (Figs. 1 and 2a). As the entrance into hibernation proceeded, the pattern of the heart

rate became more regular, and the fluctuations in blood pressure became less pronounced. Thus, when the heart temperature reached 32-21°C, the graph of highest systole and lowest diastole became much more even (Fig. 1). Slowing of the heart was accomplished both by quite evenly occurring skipped beats and by reduction of the even rate of the heart (Fig. 2b). Occasionally, while the body temperature was still dropping, the heart rate increased transiently and muscle action potentials appeared on the EKG. An increase in heart rate and a rise in blood pressure occurred at the same time (Fig. 3a). Such transient bursts

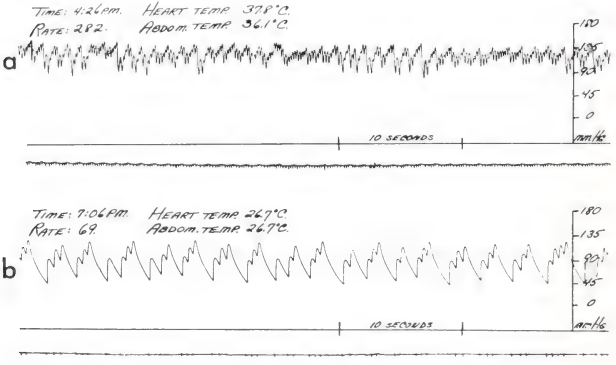

Fig. 2a. Blood pressure and EKG of same animal starting to enter hibernation. Note uneven pattern of beats. Time: 4:26 p.m. = 1½ hours on Figure 1.

Fig. 2b. Same animal later. Note even pattern of beats and skipped beats.

of activity occurred at unpredictable intervals and usually lasted too short a time to cause any difference in the decline in body temperature, but occasionally they were of longer duration and actually resulted in a brief rise in body temperature. Also as hibernation deepened, the heart rate became slower and the pulse pressure increased (compare Figs. 2a, 2b and 3a). These changes were accompanied by a slight lengthening of systole and an increasingly long diastole (compare Figs. 2 and 3, a and b).

The marked increase in the length of diastole indicated an increase in peripheral resistance as hibernation deepened. Because the systolic pressure varied greatly, it was possible to compare the rate of diastolic runoff from the same systolic pressure at all stages of the entrance into hibernation. If the increase of the angle which the diastolic pressure made with the perpendicular was plotted against temperature, the result was almost a straight line.

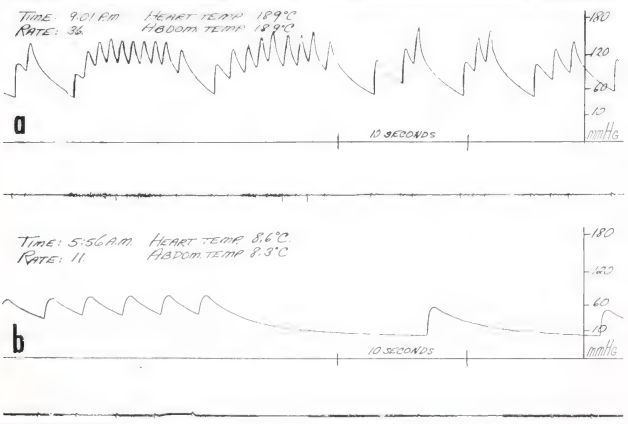

Fig. 3a. Same animal as in Figure 2, showing transient increase of heart rate at low body temperature. Note muscle action potentials on EKG.

Fig. 3b. Same animal, now in deep hibernation. Blood pressure tube slightly plugged. Blurring of EKG is electrical artifact.

In deep hibernation, two or more heart beats sometimes occurred quite close together followed by a long diastole. In such cases the second beat occurred before diastolic pressure had had time to drop markedly, and the next systolic pressure was higher than the first (Fig. 9c). This implies that there was considerable blood in the heart after the first beat. At other times the heart rate was fairly regular, though it was never absolutely even. In this case systolic pressure rose to about the same height with each beat, and the drop in pressure during the latter part of diastole was so slow, as the blood pressure approached zero, that diastolic pressure remained extremely even. In the whole series

of records the systolic pressure varied between 90 and 40 mm Hg and the diastolic between 40 and below 10 mm Hg, with heart temperatures between 5 and 8.3°C. The lowest precise record of diastolic pressure was 7 mm Hg. Very long term records of animals in hibernation were not made, but heart rates as low as three beats per minute were recorded.

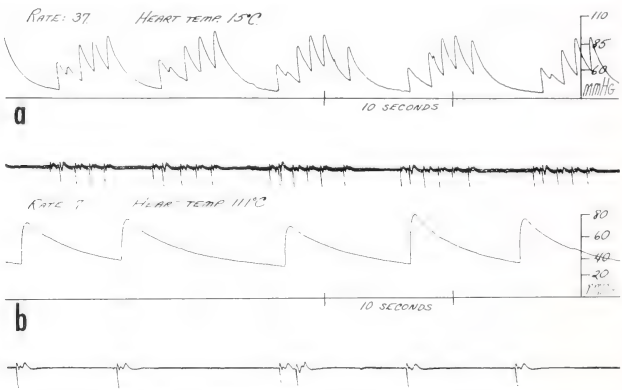

Fig. 4a. Another animal entering hibernation. Evenly occurring electrical depolarizations with little or no change in pulse pressure.

Fig. 4b. Extra systole with no change in pulse pressure. Rates measured by EKG.

Occasionally, as the animal entered hibernation, or when in deep hibernation, complete sequences of myocardial depolarizations were recorded with little or no change in pulse pressure. These sometimes occurred at fairly evenly spaced intervals (Fig. 4a) with slight changes in the configuration of the EKG, and at other times took the form of extra systoles (Fig. 4b) with no change in pulse pressure.

Complete records were obtained of animals which were stimulated to arouse from the hibernating state (Fig. 5). In some cases the animals were stimulated by poking, but in animals which were fitted only with a heart thermocouple arousal was initiated by insertion of a rectal thermocouple to a depth of 2.5 cm.

As soon as the animal was disturbed, the heart rate increased and diastole was markedly shortened. The increase of heart rate was accompanied by the appearance of muscle action potentials (Fig. 6a). These changes were often observed within two or three minutes after application of the stimulus. Later, systolic and diastolic pressures rose and the heart began to warm (Fig. 6b).

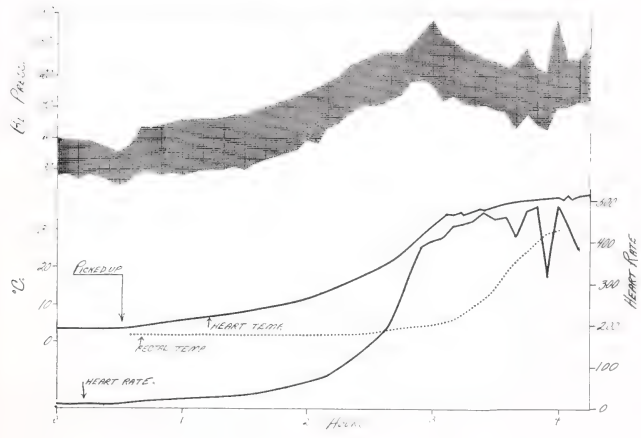

Fig. 5. Animal waking from hibernation, graphed as in Figure 1.

As arousal continued the heart rate became more rapid, the blood pressure rose and violent shivering could be seen in the anterior part of the animal. Although the plot of the highest systole and the lowest diastole does not show it clearly (Fig. 5), the pulse pressure was considerably reduced (Fig. 7a). During this time the temperature of the heart and the anterior part of the body increased rapidly, while the abdominal temperature remained nearly static (Fig. 5).

As the heart temperature approached 37°C, the abdominal temperature started to rise and the blood pressure and heart rate usually, but not invariably, dropped from the extreme heights to which they had climbed (Figs. 5, 7a and 7b). During this time diastolic runoff was more rapid, indicating a decrease in peripheral resistance. The abdominal temperature rose rapidly

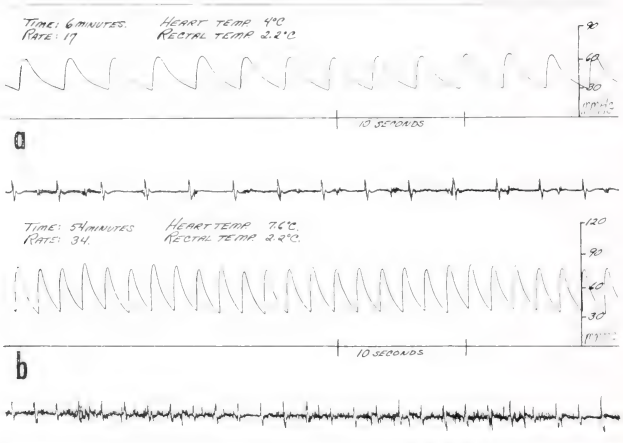

Fig. 6a. Same animal as in Figure 5. Note bursts of muscle action potentials in EKG. Time: minutes after animal was picked up.

Fig. 6b. Same animal. Note increase in systolic pressure, heart rate and muscle action potentials.

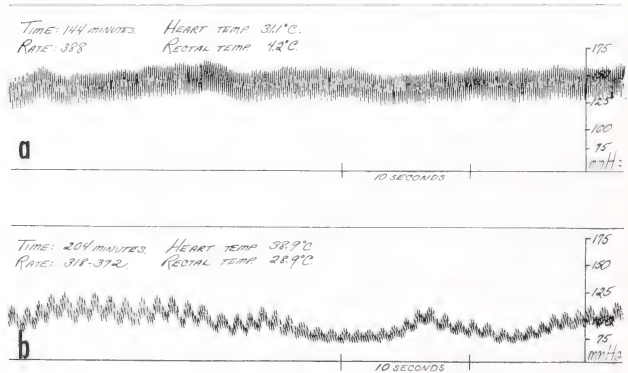

Fig. 7a. Same animal, before posterior has warmed. EKG discontinued because of fast rate and blurring by muscle action potentials.

Fig. 7b. Same animal. Posterior now warming. The even variation in blood pressure is caused by respiration.

and within 2½ to 3½ hours after the initial stimulus the animal was completely aroused. For an hour or more after this the heart and rectal temperatures averaged at least a degree above that found in the normal, awake animal.

A single record of an animal which started to arouse spontaneously at 2:30 a.m. showed the same sequence of events, with heart rate and blood pressure rising before heart temperature.

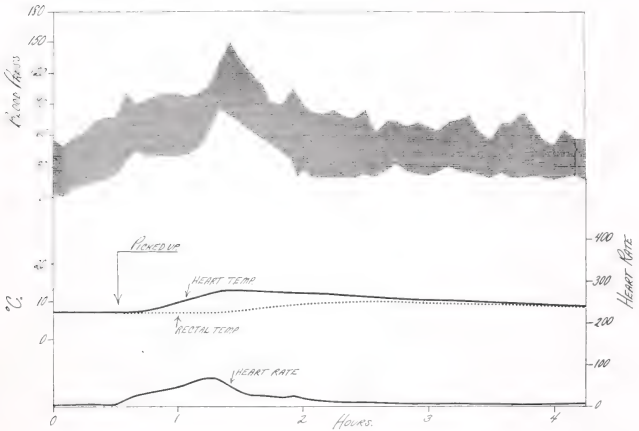

Fig. 8. Graph of partial waking and re-entrance into hibernation, as in Figure 1.

Occasionally during the winter months an animal, when stimulated during hibernation, started the arousal process, but did not complete it and returned to the hibernating state (Fig. 8). In these cases the arousal was precisely as described above, with a rapid rise in heart rate, blood pressure, temperature, and frequency of muscle action potentials. Quite suddenly, however, the heart slowed and the muscle action potentials were reduced. Peripheral resistance increased, as measured by the slope of the diastolic runoff time as described above. The blood pressure dropped, but not as rapidly as the decrease in heart rate. As the animal re-entered the hibernating state, the heart temperature declined slowly and the abdominal region, which had remained cold during the transient period of arousal, rose slowly to nearly

the temperature of the heart and then declined with the heart temperature.

Reaction to Drugs

When the experiments with drugs were begun, it was apparent that the heart of the hibernating animal was extremely sensitive to liquids introduced via the intubated aorta. As little as .07 ml physiological saline introduced quickly occasionally caused a slight transient increase in heart rate. For this reason the effect of the drugs was repeatedly checked against control injections of saline solution. Although the same drug was often used several

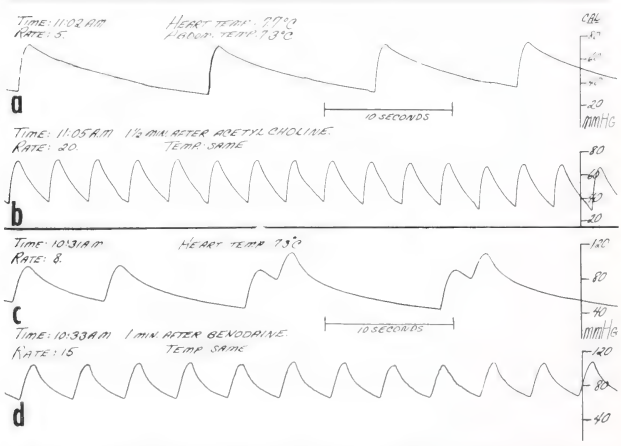

Fig. 9a. Pulse pressure in deep hibernation.

Fig. 9b. Pulse pressure after acetylcholine. Note faster diastolic runoff from slightly lower systolic pressure than in Figure 9a.

Fig. 9c. Pulse pressure in deep hibernation.

Fig. 9d. Pulse pressure after Benodaine hydrochloride. Note faster diastolic runoff from same systolic pressure.

times on a single animal, every experiment was repeated on at least two animals.

As might be expected, fairly large doses (0.15 to 0.9 mg/kg) of acetylcholine were necessary to override the presence of cholinesterase in the awake ground squirrel and cause a clear-cut effect. Once the effective dose was reached, there was a drop in

blood pressure and a compensatory increase in heart rate of 55 to 70 per cent. There was no observed bradycardia caused by this drug.

Similar doses produced a marked effect on the hibernating animals. This effect consisted of a rapid decrease in peripheral resistance coupled with a rise in heart rate. Unlike the situation in the awake animal, the systolic and diastolic blood pressure showed little or no change during this time (Figs. 9a and 9b). If the infusion of acetylcholine was continued, the heart rate increased further and the animal started the process of arousal. Although the long-term effect of acetylcholine is typical of a normal arousal, we were not able to determine whether this drug is actually the neurohumeral agent which mediates the waking process. It is possible that vasodilation and speeding of the heart were in themselves as much of a stimulus to waking as would be an externally applied physical stimulus. However, within the dose ranges used, short-term, rapid injections of acetylcholine did not cause arousal, while one sharp mechanical stimulus almost invariably produced this result.

Acetylcholine had apparently no effect on the distribution of blood once arousal was fully underway, for doses as large as 2.32 mg/kg failed to cause a change in the blood pressure or a rise in the abdominal temperature.

Benodaine hydrochloride was chosen as an adrenergic blocking agent rather than Dibenamine hydrochloride Merck or other of the better-known drugs because its effect is of short duration (Goodman and Gilman, 1958). In low doses the primary effect of this drug was a speeding of the heart and a resulting rise in blood pressure in both the awake and hibernating animals. Larger doses caused a drop in blood pressure in the awake animal although the heart rate was increased. In the hibernating animal doses of 8.5 to 25 mg/kg caused a marked decrease in diastolic runoff time along with an increase in heart rate (Figs. 9c and 9d).

Norepinephrine infused rapidly into the active ground squirrel caused a rise in blood pressure, an increase in pulse pressure and a slowing of the heart which was probably compensatory. In contrast, the effect of this drug on the hibernating animal in doses of 6 to 20 μg/kg was an increase in heart rate and pulse pressure and a rapid rise in blood pressure. Because of this rapid rise, sufficient comparative measurements of diastolic runoff time could not be made, but there was no evidence that peripheral resistance was increased by norepinephrine during hibernation. The rise in heart rate and increase in pulse pressure

alone were enough to account for the initial rise in blood pressure.

Norepinephrine at doses of 17 to 44 μg/kg was also introduced into animals during arousal, when the heart temperature had reached 37°C and the temperature of the posterior part of the body had started to rise. At this time the drug caused an immediate rise in blood pressure, and the abdominal temperature ceased rising and remained level for one minute or more. After this time the blood pressure resumed its original level and the abdominal area again started to warm. It was not possible to hold the blood pressure at the high level or stop the abdominal region from warming over long periods of time in spite of the infusion of large amounts of norepinephrine. However, the abdominal temperature could be made to rise in a step-wise fashion by periodic introductions of norepinephrine into the bloodstream.

Discussion

Records of blood pressure in any stage of hibernation are scanty, and none have been reported on mammals entering hibernation or in the undisturbed hibernating state. Dubois (1896) reported very low blood pressures after the cannulation of the carotid artery in the hibernating marmot. Blood pressure became higher as the animal aroused from the hibernating state, but Dubois did not trace the changes during the arousal process. Chao and Yeh (1951) measured the blood pressure of hibernating hedgehogs by acute cannulation of one carotid artery. The conditions of the experiment were very different from those described here, as the animals were strapped to a board throughout the experiment. These authors report that the carotid arteries were completely bloodless during hibernation, which is certainly not the case in hibernating rodents.

Chatfield and Lyman (1950) measured the blood pressure of hamsters arousing from hibernation by acutely cannulating a carotid artery. The conditions of the experiments were comparable to those reported here for the process of arousal, except that there was a time lag of 25-35 minutes to perform the cannulation. The results differ in that the rise in blood pressure was much more rapid in the hamster, but did not reach the high pressures observed in the waking ground squirrels. The observations on hamsters should be repeated using chronic intubation, not only to clarify this discrepancy, but also because the variations in the physiology of hibernation in these two species should

supply interesting comparisons on the condition of the circulation.

As far as the present results are concerned, it is apparent that there is a decrease of heart rate and blood pressure as the ground squirrel enters hibernation, and that this decrease occurs before a detectable decrease in body temperature. The equal temperatures of heart and abdomen as the animal enters the hibernating state indicate that blood flow to the anterior and posterior parts of the body is evenly distributed. As body temperature drops, peripheral resistance increases. A part of this increase in peripheral resistance is probably caused by the increased viscosity of the chilling blood. However, part of the resistance must be caused by changes in the vascular bed, for the stimulus of waking, or a vasodilatory or an adrenergic blocking drug, can quickly reduce the peripheral resistance before there is any measurable change in temperature.

The result of the increased peripheral resistance and concurrent rise in pulse pressure is that the mean blood pressure remains at remarkably high levels, even with a heart rate of only three or four beats per minute in the deeply hibernating animal. We have observed in chilled, nembutalized ground squirrels that the peripheral resistance does not rise appreciably as the animal cools, nor does the pulse pressure increase. The net result of a low systolic pressure and a rapid diastolic runoff time is a very low mean blood pressure. This may contribute to the early death of the hypothermed potential hibernator, while the animal in natural hibernation may live for many days.

The great increase in peripheral resistance with hibernation was unexpected. From our observations on the equal rate of decline of temperature in various parts of the woodchuck (Lyman, 1958), we had postulated that the animal was vasodilated as it entered hibernation. It appeared reasonable that any vasoconstriction would cause marked differences in temperature in various parts of the body as is observed in the waking hibernator. The possibility of a gradual, evenly distributed, vasoconstriction over the whole body had not even been considered. It now appears likely, however, that the hemodynamics of the ground squirrel and the closely related woodchuck during the hibernating cycle are identical, for in both animals the temperature distribution is the same on entering and waking from hibernation and in both the heart rate anticipates any change in temperature.

The hibernating thirteen-lined ground squirrel is therefore probably evenly vasoconstricted over its whole body. Prior to any measurements of blood pressure in hibernation we had suggested that the pink feet of the hibernating hamster might indicate a condition of vasodilation (Lyman and Chatfield, 1955). Although hibernation in hamsters and ground squirrels differs in many ways, an alternative explanation for the pink feet of the hamster could be the cherry-red condition of the blood during hibernation.

The presence of electrical depolarization of the heart during hibernation with little or no change in pulse pressure may be explained, in the case of extra systoles (Fig. 4b), by lack of filling time before the next beat. When depolarizations occurred at more even intervals (Fig. 4a) some effect of the deep respirations of hibernation might have reduced or obliterated the arterial pulse. On the other hand, depolarization without visible beats in isolated hearts of the ground squirrel (Landau, 1956) and hamster (Lyman and Blinks, 1959) has been reported, and complete uncoupling of the membrane phenomena from the contractile process is at least theoretically possible (Brooks *et al.*, 1955, p. 317). Whatever the explanation, it is interesting that the effective arterial pulse in hibernation can be even less than the very slow electrically measured heart rate.

When the hibernating ground squirrel starts to arouse, there is a rapid rise in heart rate and decrease in peripheral resistance. Similar results may be produced by vasodilatory drugs. In neither case is it possible to tell whether the decreased peripheral resistance causes a compensatory speeding of the heart, or whether the decrease in peripheral resistance and increase in heart rate occur at the same time. Shortly thereafter, the heart starts to warm though the posterior remains cold.

One is forced to conclude that there is a differential vasodilation in the anterior part of the body which is a vital part of the waking process. That vasodilatory drugs do not cause a warming of the posterior part of the body suggests that vascular beds of the anterior and posterior parts have different thresholds at this stage in the hibernating cycle.

Although peripheral resistance is reduced as arousal starts, the heart is able to maintain the blood pressure by increasing its rate. Indeed, as arousal progresses, the blood pressure rises and the heart rate increases, in spite of an ever-decreasing peripheral resistance. The confinement of the active circulation to the anterior part of the body results in a high blood pressure and an efficient and rapid warming of this area. In contrast, if an

active ground squirrel is given acetylcholine, the result is a drop in blood pressure even though the heart may almost double its rate. Evidently the heart cannot maintain a high blood pressure when the whole capillary bed is vasodilated at the same time.

A similar condition is found in animals during the later stages of arousal from hibernation. During this time the posterior portion of the animal is warming rapidly, indicating an unrestricted blood flow. The blood pressure, which reached its height when the anterior part of the body was still warming, now decreases because of the increase in the amount of open vascular bed. If norepinephrine is injected at this time, the blood pressure increases temporarily and the abdominal temperature remains static for a short time. One can thus produce with a vasoconstrictor the condition which obtained early in the waking process, but this condition cannot be maintained for long.

The fortuitous observations of partial arousals from hibernation fill out the general picture developed here. The arousal is normal until the heart begins to slow and the blood pressure drops. The fact that the blood pressure does not drop as fast as the heart rate indicates that peripheral resistance must now be increasing in the anterior part of the body. Since the posterior part of the body warms very slowly, circulation of blood between anterior and posterior must be sluggish, which emphasizes that the peripheral resistance in the latter must be high.

The picture during the hibernating cycle is of a circulation under remarkably precise control at all times. With our present knowledge we can only speculate about mechanisms which cause the observed changes. However, it seems clear that shifts in temperature alone do not mediate the complex interrelationships. Furthermore, whatever is controlling the heart, this organ is remarkably sensitive to stimuli at all stages of the hibernating cycle. Further study is in progress with other pharmacological agents in an attempt to clarify these problems.

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DISCUSSION FOLLOWING LYMAN'S PAPER

SOUTH inquired as to changes in blood viscosity with respect to temperature change as a contribution to the blood pressure picture in hibernation. LYMAN replied that Dr. John Pappenheimer had worked on the effect of cold on the viscosity of blood, but the results had not been published.

LANDAU remarked that diastolic pressures rather than slope of runoff curve may be more critical in estimating peripheral vascular change.

GRIFFIN inquired about a part of an electrocardiogram which seemed to be cyclic. LYMAN assured him this was an artifact periodically appearing as a 60 cycle hum. GRIFFIN

further expressed his amazement at the high peripheral resistances in hibernating animals; he wondered if the measurements reflected the difference between arterial and venous pressures across arterioles and capillaries, or a total resistance of the whole circulatory system due to high viscosity of the blood, or other causes. LYMAN replied that, though blood viscosity certainly contributed to peripheral resistance, still the rapid changes in resistance upon injection of drugs or arousal must be due to changes in the vascular bed, since they occur before a change in temperature.

BARTHOLOMEW observed that the Mohave ground squirrel has the capacity of warming the whole body at once or the front end first, depending on the circumstances. Usually the whole animal warms up at the same rate.

LYMAN suggested that if these animals were vasoconstricted throughout during arousal one could estimate the degree of vasoconstriction by giving norepinephrine and noting the change in blood pressure and peripheral resistance.

DAWE inquired as to the status of the "pink paw problem." LYMAN replied that he now believes it is due to the cherry-red color of the blood of hibernators showing through the paw surface; he no longer believes it is a vasodilation phenomenon.

BISHOP asked if the heart keeps up with body metabolism. LYMAN replied that it essentially did so, though an exact correlation has not been made. BISHOP then further remarked that a discrepancy between the rate of metabolism and the heart rate may give data usable in this context.

JOHANSSON inquired as to heart rate-peripheral resistance relationship. LYMAN said that when the peripheral resistance decreases, there is a compensatory increase in heart rate. In arousal, dilation occurs first in the anterior end of the body with a decrease in peripheral resistance there. He stated that he was not sure which came first, the increase in heart rate or the vasodilation.

MAYER said that he saw a warming of the anterior end of the Arctic ground squirrel on arousal from hibernation, followed by a steady wave of warming toward the posterior end of the body. LYMAN remarked that he did not believe it was a steady wave, but rather a sudden dilation and shunting of blood into the posterior part. This phenomenon is not as striking, he said, in

the ground squirrel as in the hamster. There is a drop in blood pressure corresponding to this opening up of the vessels and the ground squirrel warms very rapidly thereafter. MAYER stated in rebuttal that if thermocouples are placed along the length of an arousing hibernating animal they warm in sequence, not at once, and the pattern goes back over the animal as a wave of warming. LYMAN pointed out that heat conduction could mask changes in blood flow. LANDAU commented that the anterior temperature could fall when the posterior was warming.

LYMAN then asked the group if anyone knew of the possibility of other physiological conditions in which there may be complete electrical depolarization recorded without change in blood pressure. BULLARD replied that he thought such a situation occurred in certain conditions of ionic imbalance, namely calcium lack.

XIX

VASCULAR CHANGES RELATED TO HIBERNATION IN THE VESPERTILIONID BAT *MYOTIS LUCIFUGUS*[1, 2]

By Frank C. Kallen
Department of Histology and Embryology
Cornell University
Ithaca, New York

In a recent paper, Riedesel (1957) has summarized previous hematological studies on hibernating mammals and commented on the contradictory evidence for and against hemoconcentration associated with hibernation, which appears in the literature. The study presented here represents an attempt which I have made to clarify our present state of knowledge in this matter.

Plasma volume determinations have been made which give direct evidence for an over-all hemoconcentration in the hibernating little brown bat, *Myotis lucifugus*. In addition, data have been obtained relating to plasma specific gravity, hematocrit, and liver and spleen weight. Collectively, these values allow certain conclusions to be drawn concerning changes in the distribution of cellular and plasma components of the blood which are related to hibernation in this bat, and the probable effect of these changes on circulating blood volume. In light of the conflicting evidence from previous studies, it is perhaps of special interest to note that some of the results to be presented for the hibernating state would, taken by themselves, constitute evidence for a general hemodilution.

The vascular picture during hibernation has been compared with data obtained from bats under the influence of several states of activity, including arousal from hibernation and flight. This preoccupation with the active state was predicated largely by the fact that, in contrast to other hibernating mammals, resting bats have been found to lower their body temperature toward that of the environment whether they are in seasonal hibernation or not (Hock, 1951). It was therefore deemed necessary to determine whether observed changes in the circulation are dependent

[1] This work was supported by National Science Foundation grants (G-2188, G-7474) to William A. Wimsatt.

[2] The material presented in this paper is taken from a thesis to be submitted to the faculty of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

on hibernating temperatures or whether there are conditions under which the hibernating picture is duplicated over temperature ranges at which bats are commonly described as being active.

Materials and Methods

Bats for this study were captured during hibernation from caves in Pennsylvania and New Jersey. They were stored in a dark, very humid cold room at 5°C and provided with ample drinking water, but no food. Determinations were made within 3 months of capture.

"Hibernating" values were obtained on bats which had been transferred from their storage cage to individual specimen jars in which they were kept, in the dark, at 2°C for 8 to 24 hours. They were then anesthetized with ether and experiments were begun in a darkened cold room (2-3°C), using precooled apparatus. After samples had been obtained, all further procedures were carried out at room temperature.

A state of "arousal" was elicited by transferring the bats from storage to small, open-topped chambers which were located in a warm room, but were surrounded with crushed ice to cool their interiors. All experimental apparatus was chilled, but no ether was used. These were the only bats used in the study which were killed before bleeding, two by breaking their necks and the rest by freezing their heads in a dry ice-acetone mixture. The animals were bled immediately thereafter.

Bats described as "active" were brought to room temperature (21-29°C) and provided with water. After they had ceased the initial intense activity which accompanied their arousal from hibernation, the resting animals were picked up and held for a few seconds until they were actively struggling to free themselves. They were then etherized and determinations were made. Bats referred to as "excited" were treated similarly except that they were brought to a room temperature of 30°C in individual specimen jars and were encouraged to struggle for a five minute period prior to anesthesis. A final series was made up of "active" bats which had been made to fly for three to ten minutes immediately prior to the administration of ether.

Plasma volumes were determined by a modified Evans blue dye dilution procedure. Details of this technique, together with evidence for its validity and further values for active bats will be presented in a separate report (Kallen, 1960). Briefly, the method used involved the following: .040 ml of a .5 per cent

solution of T-1824 in .9 per cent saline was injected into the uropatagial vein (Grosser, 1901). After a 5-minute mixing period, a blood sample of approximately .06 ml was taken by cardiac puncture (left ventricle) from the opened chest using a hypodermic needle fitted directly to a graduated capillary tube in which the sample could be centrifuged. After (total) hematocrit was determined, a plasma sample was withdrawn, diluted and colorimetrically compared to a standard curve. Plasma volume was calculated by subtracting the volume of dye injected from the circulating fluid volume indicated by the extent of dye dilution within the animal.

No sexual or seasonal differences have been observed in any of the additional determinations which would appear to affect the plasma volumes or hematocrits to be presented here.

Samples for additional heart hematocrits, and plasma specific gravities, were obtained by the bleeding method just described. Splenic hematocrits were obtained by excising the enlarged spleens of hibernating animals in the cold room, transferring the organs to small vials and immediately collecting the blood as it drained from the spleen, with graduated capillary tubes, fitted in this case with a short length of polyethylene tubing instead of a needle. The samples were then taken from the cold room and centrifuged.

Specific gravities were determined by the falling drop method of Barbour and Hamilton (1926). The small size of the blood samples made it necessary to use a 10λ micropipette for dropping plasma. Samples for whole blood specific gravities were drawn from the heart directly into standard Lamotte dropping pipettes fitted with hypodermic needles.

All equipment used to contain blood was rinsed with an aqueous .2 per cent heparin solution and dried before use. Appropriate precautions taken to prevent evaporation and hemolysis in the extremely small blood samples included the use of parafilm as a sealing material wherever feasible.

Mean values to be presented in the text are accompanied by their standard errors. Although P values ("Student's" t test) have been calculated for all data, the values shown in Figure 1 are presented with twice their standard errors to permit graphic visualization of significant differences. Differences between means have been taken to be highly significant when they have a $P < .01$. A $P > .05$ has been assumed to be the result of random distribution. The degree of variability in individual determinations has been expressed by the use of the coefficient of variation (standard deviation x 100 / mean value).

Results and Discussion

Qualitative observations. Several obvious differences were apparent when circulatory phenomena were observed grossly in hibernating bats. The hibernating animals showed negligible patagial circulation and a markedly lowered heartbeat, suggesting a lowered blood pressure. Also, as might be expected, they were much harder to bleed. Whereas it was often possible to draw as much as .3 to .4 ml of blood from the heart of an active animal, the amount obtainable from a bat in hibernation was rarely as much as .1 ml. The only vessels in the hibernating animals which were found to bleed at all freely were the jugular veins.

The enlargement of the spleen in hibernating bats, described by previous authors (Worth, 1932; Evans, 1938; Lidicker and Davis, 1955), was also noted when the active bats used for comparison had been at room temperature for short periods, but animals which had spent 4 to 5 hours at room temperature had spleens as large as those found in hibernation.

Since ether was found not to constrict the spleen in *Myotis*, it was considered a satisfactory anesthetic for this study.

In contrast to the spleens, the livers were paler in the hibernating state, and bled less when removed. Varying degrees of reddening were typically observed as arousal began, accompanied by an extremely variable amount of spleen drainage.

Autopsy of the bats injected with T-1824 indicated a homogeneous distribution of dye in the blood of both active and hibernating bats. In contrast, the arousing animals often showed a redder color in the mesenteric vessels, or a less intense blueness in the vessels of the arms than of the legs which, in view of the uropatagial injection site, suggests that arousal in the bat is accompanied by the same preferential increase in the thoracic circulation which has been found in studies on other hibernators, notably the hamster (Lyman and Chatfield, 1955).

The blood cells exhibited differences in redness similar to those described for the blood of ground squirrels by Landau and Dawe (1958). The bright cherry-red color in blood of hibernating bats under ether was only slightly darker when hibernating animals were studied without anesthesia. During arousal, an extreme darkening was a consistent feature, while active animals showed varying degrees of brightness, but by no means to a degree comparable to the brilliance observed in the hibernating state. The volume of leucocytes in the samples was markedly

reduced during hibernation. The buffy coats averaged about .2 per cent of the blood samples taken from active bats, but only half that in hibernating animals, often being absent entirely.

Quantitative observations: liver weights. Representative determinations are those made on April 20, 1959. Four male and 4 female bats in hibernation (etherized) weighed 5.84 ±.19 g, their livers (gall bladder removed) weighed 4.91 ±.16 g/100g body weight. On the same day, a similar sample of active (etherized) bats weighed 6.05 ±.12 g, and liver weight (gall bladder removed) was 4.70 ±.12 g/100g body weight. The differences are not significant ($P>.3$ in both cases).

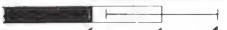
Blood specific gravities. The number of animals, and body and spleen weights were comparable to those of the animals shown in Figure 1, which were run on the same days. Blood of hibernating bats (etherized) showed a specific gravity of 1.0570 ±.0015 on Nov. 23-25, 1958, while the higher value for active bats on Nov. 16-17, 1958, was 1.0598 ±.0014, which indicates no significant difference ($P>.2$). Hematocrits and plasma specific gravities suggest that more determinations might have been in order.

Splenic hematocrit. The blood drained from the spleens of 4 male and 4 female hibernating bats (etherized) on Dec. 13-19, 1958, had a hematocrit of 75 ± 2, a value significantly higher than any heart hematocrit encountered.

Plasma volume, plasma specific gravity, spleen weight and heart hematocrit. Plasma volume or plasma specific gravity was taken concurrently with the other values on each bat. The following is a discussion of the results, which are presented in Figure 1, and to which the reader is referred.

The data for active bats injected with T-1824 indicates that hibernating bats had a significantly smaller amount of circulating plasma than was found in the active state. No mechanism which compensated for the added dye was suggested by the results, since the determined plasma volumes were the same whether .020 or .040 ml of dye were injected. In light of the specific gravities to be discussed, the slightly greater variability encountered in the series injected with .020 ml might be attributable to the disturbance caused in the storage cage by removing bats for the .040 ml injections on the previous day. By the same reasoning, the active animals injected must also be considered as having been recently disturbed during hibernation. Notice that, although the active bats had been at room temperature for periods ranging from 30 minutes to 8 hours, their plasma volumes were

FIG 1 CHANGES IN SPLEEN WEIGHT, HEMATOCRIT, AND PLASMA OF *MYOTIS LUCIFUGUS*.



* Bled before removal of spleen
† No ether used

BATS INJECTED WITH T-1824

STATE OF ACTIVITY	NOS. ♂	NOS. ♀	DATES	REMARKS	BAT WT, G CV, % 5 15 5.75 7.25	SPLEEN WT., G/100 G CV, % 2.5 7.5 .300 .900	HEART HCT. (DYED BLOOD) CV, % 5 15 45.0 55.0	PLASMA VOL., ML/100 G CV, % 10 30 5.0 7.0
HIBER-NATION	9		Apr 16-21, '58	*				
	9		Apr 22, '58	.020 ml. dye*				
AROUSAL	8		Feb-Apr, '56 & '58	*†		INCONSISTENT		
ACTIVE	9		Apr 23-24, '58	act 1/2-6 hr *				

BATS ON WHICH PLASMA SPECIF. GRAVS. (T:T) WERE TAKEN.

STATE OF ACTIVITY	NOS. ♂	NOS. ♀	DATES	REMARKS	BAT WT, G CV, % 5 15 5.75 7.25	SPLEEN WT., G/100 G CV, % 2.5 7.5 .300 .900	HEART HCT. CV, % 5 15 45.0 55.0	SPECIF. GRAV. PLASMA CV, % 20 40 1.0250 1.0290
HIBER-NATION	4	4	Nov. 23-25, '58	*				
	4	4	Nov-Dec., '58					
	4	4	Dec. 20-22, '58	†				
ACTIVE	4	4	Nov 16-17 '58	*				
EXCITED	4	4	Jan. 15-19, '59	act. 2-3 hr *				
	4	4	Jan. 15-19, '59	act. 2-3 hr				
FLOWN	4	4	Jan. 16-18, '59					
EXCITED (Pool of 9♂, 9♀)	4	4	Jan. 12, '59					
	4	4	Jan. 13-14, '59					
	4	4	Jan. 12-14, '59	act. 1-2 hr				
	4	4	Jan. 12-14, '59	act. 4-5 hr				
	4	4	Jan. 12-15, '59	large spleens				
	4	4	Jan. 12-15, '59	small spleens				

remarkably consistent, suggesting no great fluctuation in plasma levels. This finding was confirmed by incorporating additional determinations in a comparison of values for 10 bats active for 30 minutes to 1 hour (7.2 ±.3 ml/100g body wt.) with those for 11 bats active for 1 to 3 hours (7.2 ± .2 ml/100g) and those for 9 bats active for 3 to 8 hours (6.7 ±.2 ml/100g). These means do not differ significantly ($P>.1$ in all cases).

The determinations for bats in a state of arousal clearly show that a rise in plasma volume had been initiated in the 5 to 10 minutes which had elapsed since the animals had been in hibernation. Indeed, the values shown are probably lower than the actual volumes, owing to the dye localizations previously mentioned, although the extent of this localization was not sufficient to suggest that actual plasma volumes were yet equal to those found in the active state. Spleens ranged from moderately full to completely empty, and were far too inconsistent in size to suggest any worthwhile quantitative treatment under the conditions of this experiment. The rising plasma levels and injected dye would both be expected to depress hematocrit, yet hematocrits during arousal, though extremely variable, were at the highest level encountered in the study, suggesting a mobilization of cells in the thoracic circulation at this time.

Comparison of spleens in all hibernating bats studied shows a significant decrease in weight accompanying dye injection, which suggests increased blood pressure as a factor in splenic evacuation.

Turning now to the determinations made on undyed bats in November and December, a significant increase in spleen weight during hibernation can be seen (this active series had been at room temperature for 30 minutes to 4 hours). Spleens taken from a comparable series of active bats, which had not first been bled, weighed .401 ±.047 g/100g body weight, a figure comparable to that found for the excited animals shown immediately below which were splenectomized before bleeding in January. Thus, although loss of blood is accompanied by a significant drainage of the spleen in active bats, bleeding alone cannot account for the size difference between the active and hibernating states.

Hibernating bats under ether had significantly lower hematocrits than (etherized) active ones, which would imply a hemodilution in the heart region. A dilution of the plasma itself was implied in the significant drop in the corresponding plasma specific gravity. These specific gravity differences became less

marked when the spleen was removed before bleeding the (etherized) hibernating animals ($P>.01$) and disappeared ($P>.1$) when the spleen was removed and blood subsequently taken from hibernating animals on which no ether was used. The progressively increasing coefficients of variation reflect the greater variability in plasma specific gravities which might be expected in the hearts of more disturbed animals if plasma proteins had not been uniformly distributed in the resting state. Hibernating hematocrits underwent similar changes, although they never approximated those of the active bats (in no case was $P>.05$). In the case of the splenic weights, however, the least variation was found in the animals which had not been etherized. This suggests a relatively constant amount of blood trapped in the splenic pulp in a manner which prevented its release by any mechanism as simple as the squeezing action of the abdominal skeletal muscles which was observed in the absence of ether.

When comparing the first two groups of excited bats which were alike except that one series was bled before splenectomy and the other after, it is seen that spleen sizes are comparable to those found in active bats in December. Hematocrits have dropped to slightly lower levels (when compared to those in the hibernating state), and plasma specific gravities no longer differ significantly from those found during hibernation. This drop was even more consistent in bats which had been flown. Although bleeding resulted in a significant drainage of the spleen, no significant change in heart hematocrit was observed, which may reflect a temporary trapping of blood from the spleen in the liver.

The data from the pool of 9 males and 9 females shows no significant difference in splenic weight or hematocrit between bats which had been left undisturbed for a matter of weeks (Jan. 12) and those which had been disturbed in storage the day before (Jan. 13-14). The plasma specific gravity of the undisturbed animals, however, is high enough to differ at a suggestive level ($P<.05$) from the lowest hibernating value. This makes it necessary to call attention to the dates (Nov. 16-17) on which the active determinations were made; these bats had been left undisturbed for several weeks. Since the experience in our laboratory has been that bats need ample water and high humidity to survive hibernation at all, the most reasonable interpretation of the high specific gravities which have been encountered appears to be that bats drink less frequently when hibernation is undisturbed, undergo some dehydration, and have less body

fluid available to be contributed to the plasma when the animals increase plasma volume upon entering the active state. This would imply that the increase in plasma volume may not always be of the magnitude observed in the dye studies, and that, although significant differences in hematocrit and plasma specific gravity may be expected after undisturbed hibernating bats have become active, these differences might lessen or disappear if the hibernating animals had recently awakened to drink. In all other cases, reasonably uniform average values for plasma density were maintained, although they were not uniformly consistent. Since greater variability appears not to be associated with lower specific gravity, it cannot be explained adequately by assuming nothing more than sporadic dilution of heart blood by lymph from the thoracic ducts; the possibility of a localization of plasma proteins somewhere in the circulation again suggests itself.

When excited bats which have been at 30°C for 1 to 2 hours are compared to those which have presumably come closer to a resting state after 4 to 5 hours, the larger ($P<.05$) spleens in the latter more closely approximate the hibernating condition. This suggests that more critical studies might uncover a reversion toward the hibernating picture in bats at rest at room temperatures. The high plasma specific gravity in the active bats, for example, may prove to be not entirely due to dehydration, but also to a lower plasma volume than that found in more excited bats. This tendency would probably not be detectable by a dye dilution procedure, since the injection and mixing period probably serve as excitatory stimuli in themselves.

A final point in regard to specific gravity is that the decreased plasma volume during hibernation is not reflected in a higher density of either blood or plasma in the heart, which suggests that the system is taking steps to prevent extra work by the heart at a time when the blood is already more viscous as a result of cold.

When the animals are segregated on the basis of spleen size (large vs. small spleens), a significant ebb and rise in hematocrit is indicated, which has been obscured in most cases by the rhythmic activity of the spleen. This finding, in conjunction with the high hematocrit of splenic blood, clearly establishes the spleen as a source of blood cells for the rest of the circulation, although the extent of its contribution has yet to be discussed.

Probable changes in circulating blood volume. Since independent measurements of blood cell volumes were not made, directly measured blood volumes cannot be given. The rapid rate of the plasma volume changes, however, together with Worth's (1932) observations on the generally quiescent state of hemopoiesis during hibernation in the bat (only the spleen and lymph nodes were found to be active) make it reasonable to assume that a constant volume of blood cells is available within the animal while these changes take place. Relative comparisons are therefore possible to a degree.

For example, if we calculate blood volumes for the hibernating and active bats injected with .040 ml of T-1824 on the basis of plasma volume and observed hematocrit (subtracting the amount of dye injected and assuming approximately 4 per cent of the hematocrit cell column to be trapped plasma) using a ratio F *cells* (total body hematocrit/heart hematocrit) of 1 in each case, hibernating blood and plasma volumes are .550 ml and .300 ml respectively, while those for active animals are .809 ml and .400 ml. Hibernating bats thus appear to have .250 ml of cells and active bats .409 ml of cells; a difference of .159 ml must be accounted for.

Observed splenic weight loss was .038 g (based on the extremes in mean splenic weights of hibernating and active undyed bats of the Nov.-Dec. series), which on the basis of the data presented would represent only .026 ml of blood cells. Even if we made the liberal assumption that these spleens actually had contained .04 ml of "extra" cells, none of which had been accounted for in the calculated hibernating cell volume, the splenic contribution could account for only about one third of the discrepancy.

No other region of red cell concentration remotely comparable to the spleen has been found in the bat, for Worth's (1932) observations would discount bone marrow, and the liver has been discounted here. We must assume a more general shift of blood cells away from the heart region during hibernation. Dodgen and Blood's (1956) estimate of .5 ml for bat blood volume is unfortunately based on "unpublished data" with no description of method of determination; however, they were apparently referring to the volume in *Myotis lucifugus*. Their value is in close agreement with the volume of .550 ml calculated here for the hibernating bat, which suggests that the true cell volume may be as low as .250 ml, implying a bat F *cells* ratio near 1 in hibernation and near .6 during activity. Nevertheless, work has

been initiated in our laboratory to determine cell volumes directly, for the F *cells* ratio thus implied for the active bat is extremely low. Reeve *et al* (1953) have found F *cells* of about 1 in normal dogs, which rose to about 1.1 in normal dogs anesthetized with pentobarbital sodium, and fell to about .9 in splenectomized dogs.[3] At present, it seems most reasonable to conclude that since fewer red cells are needed for respiratory exchange during hibernation, as evidenced by their color, a large proportion of them stagnates in the sluggish general circulation, away from the heart, and that, conversely, "extra" plasma is present in the capillary beds of the active circulation, but not to the extent of .6 F *cells* ratio. The amount of relative change in these ratios, however, emphasizes the inadequacy of hematocrit determinations as a source of information on circulatory volume change during hibernation. Specific gravities also appear to be affected by varying distribution of blood components, even when the plasma alone is considered. The contradictory results of other studies suggest that these phenomena are not peculiar to bats. An increased emphasis on direct measurements of volumes appears necessary, therefore, not only for its own sake, but for proper evaluation of metabolic changes in general which are reflected in concentration changes of blood constituents during hibernation.

Summary

A significant decrease in plasma volume during hibernation was found in the little brown bat, *Myotis lucifugus*, by using a modified Evans blue dye dilution procedure. This decrease was not reflected in higher hematocrits or higher specific gravities of blood or plasma taken from the heart in other bats, which suggests a redistribution of cellular and plasma protein components of the blood to ease the work of the heart during hibernation. A mobilization of blood cells in the thoracic region during arousal was suggested by the high mean hematocrit observed; dye studies indicated a preferential thoracic circulation at this time. A significant rise in hematocrit and plasma specific gravity after arousal was noted in bats which had been in relatively undisturbed hibernation, suggesting a dehydration when compared to bats which had been disturbed recently and,

[3] These authors are referring to ratios based on venous hematocrit rather than heart hematocrit. However, additional data (Kallen, 1960) indicates that mean venous hematocrit (uropatagial vein) does not differ significantly from mean heart hematocrit in samplings of active bats injected with T-1824, although individual variation is masking a slightly higher venous value.

presumably, had taken water while aroused. Part of this rise, however, might also be attributed to a reversion toward the hibernating blood picture whenever bats are at rest, even at room temperatures, since spleens enlarge to a size previously described only during hibernation when bats have been at rest at 30°C for 4 to 5 hours. The spleen was found to contribute significantly to circulating cell volume and appears to be the only organ which was doing so. However, the volume of cells in the spleen is inadequate to account for the extra cell volume implied by calculating cell volume from plasma volume and heart hematocrit when a constant F *cells* is assumed. A more general concentration of cells in the peripheral circulation is suggested during hibernation, and the reverse during activity.

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DISCUSSION FOLLOWING KALLEN'S PAPER

BULLARD asked if the mixing time had been standardized for each level of animal activity. KALLEN replied that, using a 5-minute mixing time, he had taken two or more blood samples from individual active bats, one sample from a wing vein and one or more from the heart. The similarity of plasma-dye concentrations in these samples had appeared indicative of complete mixing. This procedure had proved impractical in the case of hibernating animals owing to the difficulty of drawing blood from the lowered peripheral circulation. However, routine autopsies consistently showed an apparently homogeneous distribution of the intensely colored dye in both active and hibernating animals and he (KALLEN) thought it most likely that mixing had been complete in hibernating bats, especially since localization of dye was readily observable during states of arousal. BULLARD asked if mixing time had been changed with lowered body temperatures, and KALLEN replied that it had been kept constant for all states of activity.

ADOLPH noted that the animals appeared to lose a considerable volume of blood during hibernation — was this due to dehydration? KALLEN replied that, although some dehydration appeared to occur during prolonged hibernation, most of the water lost by the blood appeared to remain within the animal since higher plasma volumes were observed when hibernating bats were brought to a state of relatively high activity at room temperature without having drunk. ADOLPH asked how long the torpid state had persisted before the animals were used. KALLEN said this was difficult to assess, although the data presented for "disturbed" and "undisturbed" bats suggested that it had persisted for some time when their storage cage was left

unopened. However, in accord with HOCK'S earlier evaluations of time required for the bat to attain a minimum metabolic rate at low temperatures, all animals used had been kept at 2°C for at least 8 hours immediately before determinations were made. ADOLPH then asked how long the animals had been kept in the cold. KALLEN replied that the particular animals used had been hibernating naturally in caves for at least two months, and kept in the cold room for not longer than three months after their capture.

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PERIPHERAL NERVE FUNCTION AND HIBERNATION IN THE THIRTEEN-LINED GROUND SQUIRREL, *SPERMOPHILUS TRIDECEMLINEATUS*[1]

By Theodore H. Kehl and Peter Morrison
Departments of Zoology and Physiology
University of Wisconsin
Madison, Wisconsin

If hibernating mammals are to remain responsive to environmental stimuli throughout hibernation, their nervous pathways must remain functional at body temperatures so low that conduction would be blocked in most non-hibernating mammals. It has been shown by Chatfield *et al.* (1948) that the peripheral nerve in one hibernator, the golden hamster, is functional at somewhat lower temperatures than in the albino rat, a non-hibernator. Such an adaptation should have definite survival value, allowing a hibernating animal to respond to stimulation during hibernation. One might also expect to see differences in nerve conduction between hibernating and active phases in the life of a hibernator, but such were not found in the hamster. However, the hamster might be termed a "permissive" hibernator inasmuch as it stores food and does not necessarily hibernate during the winter; it is, moreover, native to lower latitudes. One might expect further specialization in more northern species which invariably hibernate. This study describes peripheral nerve function in such an "obligate" hibernator, the thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*), in terms of the temperature functions of conduction, excitation and refractory period.

Materials and Methods

These experiments were carried out during 1957 and 1958. The thirteen-lined ground squirrels were trapped in the summer for use during the following year. Although the ages of the animals were unknown, a great number were probably from spring litters of the year of capture. Experiments on active animals were carried out during June and July. During the

[1] This research was supported by the Wisconsin Alumni Research Foundation, and U.S.P.H. grant no. H-2095.

hibernating season (November through March) the animals were housed in a "hibernaculum," a special bank of cages providing isolation from sound and light and in which the temperature was maintained at 5-10° by the circulation of refrigerated water through hollow walls. Arrangements were made to feed the animals in measurable amounts without disturbing or altering the environment. When the consumption of food and water had dropped to zero, one could be certain that the animal was no longer active and no longer regulating its body temperature. At intervals of 1 to 3 weeks it was observed that food and water had been consumed over periods of one to two days, thus indicating that the animal had awakened temporarily. During most of the intervening fasting period the animal was surely in hibernation, but it is quite possible that partial awakening without food or water consumption had occurred during these periods.

Animals were taken for experiments at various times during the hibernating cycle: at three days of hibernation, at 12 days of hibernation, and then after 32 days total hibernation. To define transitional stages, two additional groups of animals were studied: animals which had been hibernating but were stimulated to arouse until the body temperature had reached 20°C, and animals which had been hibernating in the cold but had spontaneously become active and remained so for some weeks. Finally, a group of animals were examined which had not been subjected to a cold environment but had become lethargic at room temperature during the winter (ordinary hibernating) season.

The sciatic nerve, used in these experiments, was prepared as follows: removed from the hibernaculum and weighed, the animal was immediately decapitated and the body temperature taken; the carcass was dissected at 5°C in a cold room. With a blunt glass probe the sciatic nerve was exposed from its insertion in the gastrocnemius to its origin at the spinal roots. All side branches were severed, the proximal and distal ends were tied off and the nerve cut free. During dissection the nerve was swabbed with cold Locke's solution.

The freed nerve was placed in a nerve chamber (Plate) so that one end lay between two stimulating electrodes and the remainder rested on a series of pickup electrodes spaced 5 mm apart. With three selector switches any pair of pickup electrodes could be chosen for recording and any third for a ground. The electrodes were enclosed in a lucite housing through which water from a constant temperature bath circulated. Between measurements the chamber was flooded with Locke's solution.

During a typical experiment the nerve was placed in the nerve chamber at 5°C and allowed to equilibrate for 10 minutes before the measurements for this temperature were made. The constant temperature bath and the nerve chamber were then warmed through 2.5°C and measurements made again after a 10-minute equilibration period. In this way measurements were made every 2.5 degrees up to 20°C, then every 5 degrees from 20 to 35°C, and finally at 37°C.

A Textronix,[2] Model 532, oscilloscope with a DuMont oscilloscope camera[3] was used to record the action potential photographically. The stimulus was produced by a Grass stimulator (Model S4C)[4] through a stimulus-isolation unit.

In measuring conduction velocity a maximal stimulus of 0.1 msec duration was used. This represents the lowest stimulus that will just excite all the fibers in the nerve. Excitability was determined at stimulus durations of 0.1 and 0.01 msec. The threshold was measured at the maximum sensitivity of the oscilloscope (20cm/mV) by observing the voltage at which the action potential just disappeared.

The refractory period was measured by shortening the time between two successive stimuli (0.1 msec) until the action potential which resulted from the second stimulus showed a decrease in spike height. This interval is a relative refractory period and relates to the behavior of the least excitable fiber groups. The time between two stimuli at which the second action potential just disappeared was also measured. This is taken as an absolute refractory period and is dependent upon the most excitable group of fibers. The stimulus-strength for the measurement of absolute refractory period was about 10 times the threshold.

Results

Conduction velocity. Conduction velocities from a typical experiment are plotted in Figure 1. These data show a linear relationship of conduction velocity with temperature which was characteristically seen in this study. This particular experiment was unusual in providing two measurable spikes. Here, the faster fiber-group is taken to be the alpha, with velocity at 37°C of 100 M/sec. The slower fiber-group with velocity of 30 M/sec

[2] Textronix Inc., Portland, Oregon.

[3] Allen B. DuMont Laboratories Inc., Clifton, New Jersey.

[4] Grass Instrument Co., Quincy, Massachusetts.

at 37°C was judged to be the beta. Only in animals which have just entered hibernation was it possible to obtain clear-cut responses from the slower fibers. Although a beta spike could be seen in nerves from other groups, it was too close to the alpha spike to be accurately measured.

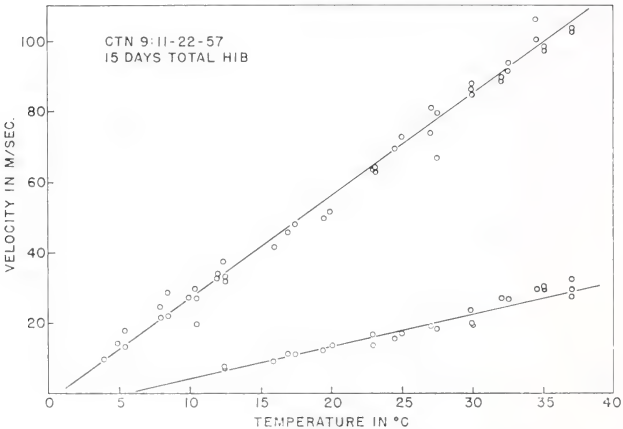

Fig. 1. Conduction velocity as a function of temperature. Both alpha (upper group) and beta (lower group) components are shown. This run is an example of an animal in the transitional stage.

Figure 2 demonstrates the stability of the isolated nerve preparation by comparing measurements made at 2, 9, 24, 48 and 72 hours after decapitation. There was excellent reproducibility over this entire period, during which the nerve was held at 5°C, with, however, some decrease in excitability even after 24 hours.

The two parameters describing a linear function are the slope (dV/dT) and the intercept (at V=0). The average values for these conduction-velocity increments and intercepts in the several groups of animals are tabulated in Table I and plotted in Figure 3. It may be seen that the conduction-velocity increment for the active animal is considerably greater and the intercept is higher than for the hibernating animal. The intercept values for the active ground squirrels are the same as those noted by Chatfield *et al.* (1948) for the hamster. The slopes appear to be of

the same order of magnitude, but unfortunately cannot be exactly compared. Accordingly, in the thirteen-lined ground squirrel there is an adaptation during hibernation to facilitate conduction at lower temperatures than would be possible in the active ground squirrel.

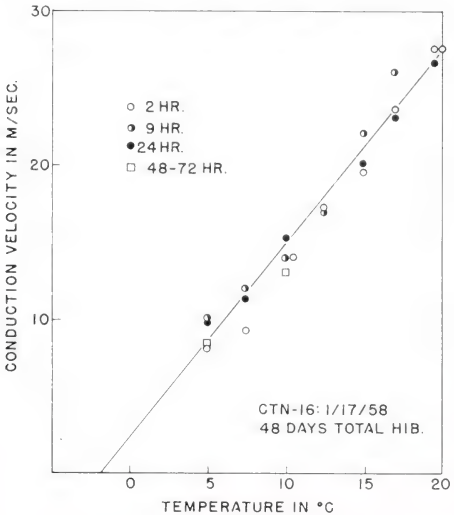

Fig. 2. Conduction velocity versus temperature in a series of measurements extending over a period of 72 hours.

The average curves for animals that had just entered hibernation (3 days and 12 days) (Fig. 3) show a progressive lowering of the intercept with no change in slope. Consequently, the nerve has maintained the same ability to respond to changes in temperature, and yet has adjusted so as to function at temperatures that would otherwise block conduction. As hibernation is extended from 12 to 32 days or longer, the slope is reduced by about one-half its former value with little change in intercept.

In animals sacrificed during the awakening process (T_B=20°C) the nerve maintains unchanged its ability to conduct at low temperatures, but the conduction-velocity increment has increased during this brief period (½ hour) to a value halfway

between those for the active and hibernating animals. The average curve for the animals that had awakened spontaneously from hibernation and were active at 5°C is essentially identical to that for the active animals.

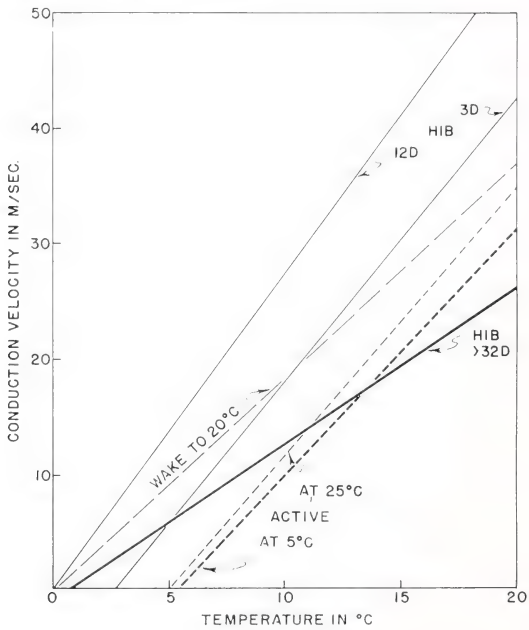

Fig. 3. Mean curves for conduction velocity versus temperature for the various groups of animals used in this study.

Excitability. The reciprocal of the threshold voltage may be used as a measure of excitability. Our data show that a linear relationship exists between excitability so measured and temperature (Fig. 4.) Mean values for slopes and intercepts are given in Table II and plotted in Figure 5. The excitability increment for animals hibernating for a long time (32 days) is lower than for active animals and the intercept is also lower for hibernating animals. Thus, the nerve of a hibernating ground squirrel is

excitable at lower temperatures than the nerve of an active ground squirrel; any change in temperature will have a smaller effect on it. The changes in slope and intercept for excitability are exactly analogous to those found for conduction-velocity.

TABLE I

Slopes and Intercepts of the Temperature Functions of Conduction (M/sec. vs. °C) in the Sciatic Nerve of the 13-lined Ground Squirrel under Various Conditions.

		Slope in M/sec^{-1} °C^{-1}		Intercept in °C	
Group	Number	Mean	S.E.	Mean	S.E.
Active at 25°C (summer)	8	2.30	.16	4.8	.68
Active at 5°C	3	2.29		6.0	
Active at 25°C (winter)	3	1.84		4.0	
Hib. at 5°C					
3 days	2	2.38		2.6	
12 days	2	2.71		0.0	
32 days	19	1.43	.06	1.8	.33
Hib. at 25°C	6	1.61	.13	2.7	.57
Awake 5–20°C	3	2.29		6.0	

	Conduction Velocity	Slope T-Value	Intercept T-Value
I.	Active vs. hib. both at 25°	2.1	4.9
II.	Hib. 5° vs. awake to 20°	0.4	0.4
III.	Hib. at 25° vs. active at 5°	1.3	0.3
IV.	Active at 25° vs. active at 5°	1.8	3.0
V.	Winter active vs. summer active	2.9	

If one compares the intermediate stages, quite a different picture appears (Fig. 5). In animals just entering hibernation, after 3 days the average slope and intercept differed but little from the active animal, but after 12 days the average slope and

increment were at the hibernating level (32 days). The lowest intercepts were observed in animals which had become active at 5°C following hibernation. The curve obtained for animals in the process of arousal was essentially the same as the curve for active animals at 5°C.

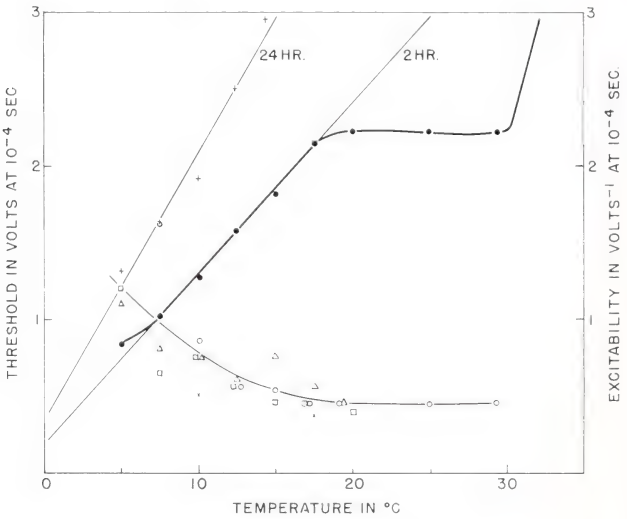

Fig. 4. Representative results of excitability versus temperature indicating reciprocal threshold voltage 2 hours after the animal was decapitated and 24 hours after decapitation. Other symbols are thresholds measured over a period of 48 hours. The discontinuity at 17.5°C was seen in several other experiments but had no correlation with the state of the animal. Only the linear portion of the plot to 20°C was used to derive the mean.

Refractory period. The refractory period represents a measure of the time required for completion of some recovery process in the nerve fiber. Its reciprocal, which is proportional to the rate of this reaction, was not found to be a linear function of temperature, as were conduction velocity and excitability (Fig. 6). When the logarithm of the reciprocal of refractory period was plotted against temperature, a linear function resulted. There was a significant shift in the Q_{10} of recovery in the hibernating

ground squirrel nerve as compared to that in the active (summer) animal (4.0 to 3.1) and this change was not shared by any other group (Table III). Amberson (1930) gave a value of 3.0 for the Q_{10} of the absolute refractory period in the sciatic nerve of *Rana esculenta*. It may be significant that high tempera-

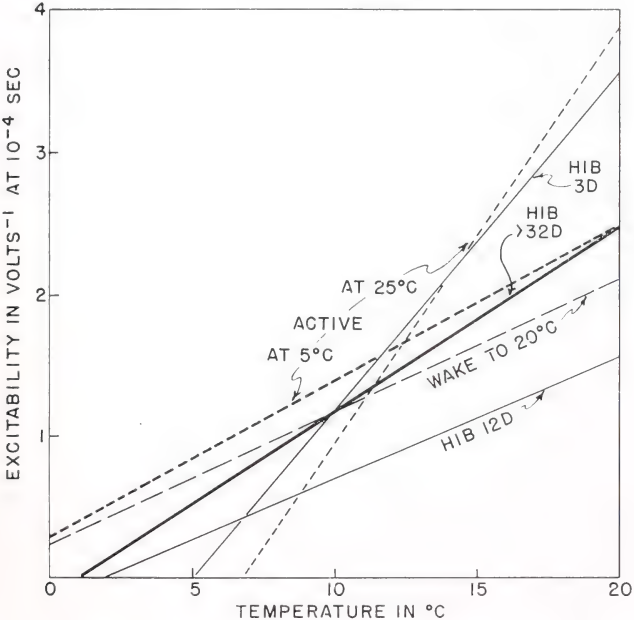

Fig. 5. Mean curves for excitability versus temperature for various groups of animals used in this study. Animals "active at 5°C" had previously hibernated.

ture coefficients are also observed in the overall changes of metabolic rate (Kayser, 1940; Hock, 1951). On the other hand, much lower temperature coefficients are found for isolated tissues including brain (South, 1958; Meyer and Morrison, 1960).

The linear relationships between temperature and conduction or excitability suggest "physical changes" in the nerve, perhaps

not inappropriately in these instances. However, the logarithmic function describing the refractory period is suggestive of some changed "chemical" process supplying energy for recovery. Hence, a van't Hoff or Arrhenius function is in order. The higher Q_{10} exhibited by the nerve of the hibernating ground squirrel would suggest that this nerve is better able to respond metabolically to changes in temperature.

TABLE II

Slopes and Intercepts of the Temperature Function of Excitability in the Sciatic Nerve of the 13-lined Ground Squirrel under Various Conditions.

		Slope in V^{-1} °C^{-1}		Intercept in °C	
Group	Number	Mean	S.E.	Mean	S.E.
Active at 25°C (summer)	6	0.29	.063	6.8	.59
Active at 25°C (winter)	4	0.18	.033	2.9	1.16
Active at 5°C	2	0.106	.81	0.3	2.1
Hib.					
3 days	2	0.25		5.0	
12 days	2	0.10		3.0	
32 days	15	0.13	.19	0.3	.46
Hib. at 25°C	6	0.14	.03	1.0	1.5
Awake to 20°C	4	0.107	.057	0.9	1.5

	Slope T-Value	Intercept T-Value
I. Active vs. hib. both at 25°	3.5	3.3
II. Hib. 5° vs. awake to 20°	2.3	1.6
III. Hib. at 25° vs. active at 5°	0.3	0.4

Discussion

Conduction and excitation in the sciatic nerve of the thirteen-lined ground squirrel have been shown to be linear functions of temperature. Such functions are suggestive of a "physically"

dependent reaction, whereas the logarithmic function describing the refractory period is suggestive of a chemical reaction perhaps supplying energy for recovery. Amberson (1930) showed such a function in the sciatic nerve of the frog. His value for the Q_{10} of the reciprocal refractory period (3.0) was identical to that found here for the active ground squirrel.

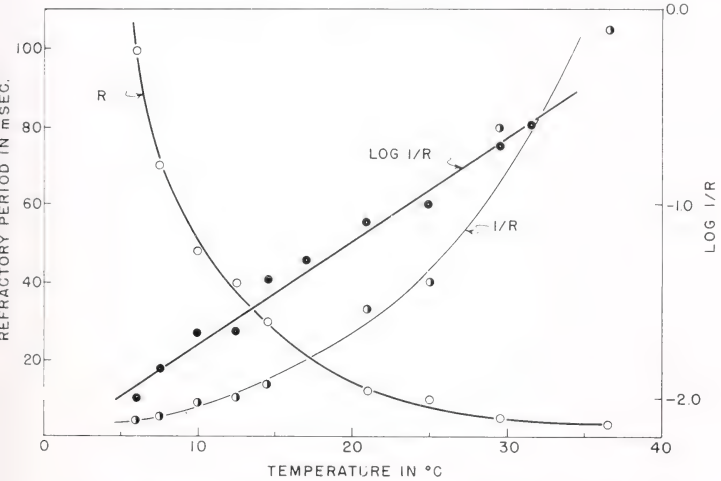

Fig. 6. Refractory period versus temperature for the various groups of animals used in this study. Open circles show direct plot (in msec), half circles show reciprocal plot (in $msec^{-1}$) and solid circles show the log reciprocal plot (log $msec^{-1}$).

The normal ground squirrel nerve has a temperature function quite similar to that obtained by Chatfield *et al.* (1948) for the golden hamster. However, these investigators found no difference between hibernating and active hamsters, and compared the data from the hamster nerve with data from the albino rat nerve. One must keep in mind that the ground squirrel is an "obligate" hibernator and not a "permissive" hibernator as is the hamster. Therefore, one may expect to find physiological adaptation occurring in "obligate" hibernating animals which may not be demonstrable in the "permissive" hibernators.

TABLE III

Temperature Coefficients for Recovery of Sciatic Nerve in the 13-lined Ground Squirrel.

	Relative Refractory[1] Period Q_{10}			Absolute Refractory[2] Period Q_{10}		
Group	Number	Mean	S.E.	Number	Mean	S.E.
Active at 25°C (summer)	1	3.10				
Active at 25°C (winter)	7	3.10	.26	4	3.08	.27
Active at 5°C	4	3.21	.36			
Hib. at 25°C	6	3.12	.09	6	3.32	.45
Hib. at 5°C	12	3.95	.27	4	4.29	1.33
Awake 5–20°C	4	3.09	.41			

[1] Reciprocal relative refractory period. Represents the least excitable fiber group; time between pairs of maximal stimuli to provide measurable diminution of second spike.

[2] Absolute refractory period. Most excitable fiber group; time between pairs of maximal stimuli to just eliminate second spike.

Significance: Active at 25° vs. hib. at 5° : T = 2.5
Hib. at 25° vs. hib at 5° : T = 3.0

"Transitional" Properties

The linear form of the temperature functions and their changes in slope and intercept showed close analogy between conduction and excitation in the active summer and hibernating winter animals. This suggests some common underlying activity, and indeed conduction may be considered as propagated excitation. While it would be attractive to ascribe these functional changes to some single underlying condition, examination of results in the transitional stages shows that the situation is more complex. Thus, although usually correlated, a shift in intercept (rate constant) was not always accompanied by a change in slope (temperature coefficient) and conversely. Further, in transitional stages, observed changes in the conduction function did not always parallel the changes in excitability.

The shift in the Q_{10} of recovery in the hibernating (32 days) ground squirrel nerve as compared to the active ground squirrel nerve (4.0 vs. 3.1) has the opposite sign from the Q_{10}s for conduction and excitation, i.e., the temperature coefficient is increased in hibernation rather than decreased. However, this change in recovery function corresponds to the change in temperature function seen for tissue respiration (Meyer and Morrison, 1960).

It may be significant that high temperature coefficients are also observed in the overall changes of metabolic rate seen in hibernators (Kayser, 1940; Hock, 1951). On the other hand, much lower coefficients are found for isolated tissues including brain (South, 1958; Meyer and Morrison, 1960).

The underlying causes of these modifications in nerve function are of much interest. It is known that in the hibernating mammal there is a general involution of the endocrine glands (Johnson, 1931; Kayser, 1940), so that some hormonal influence may be involved either directly or indirectly. Such a notion is supported by the fact that the endocrine involution is seasonal, that is, it precedes actual hibernation. Ground squirrels which had not entered hibernation during the winter showed a modification in conduction-velocity increment and excitability increment similar to that seen in hibernating animals (Tables I and II; active at 25° (winter) vs. hib. at 5° (32 days)). More direct evidence of hormonal involvement in nerve function is provided by experiments on nerves from hypophysectomized albino rats (unpublished observations with R. K. Meyer) which show lower slopes and intercepts for both conduction and excitability functions. Since this transformation exactly parallels the change in the hibernating ground squirrel, it strongly supports the concept of a control of axonal function through hormonal levels mediated through the hypophysis.

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DISCUSSION FOLLOWING KEHL'S PAPER

GRIFFIN remarked that KEHL was in effect measuring a number of synchronized potentials in a nerve trunk. KEHL agreed, and pointed out that the refractoriness of the most excitable fibers was determined by a two-stimulus sequence using a stimulus at ten times threshold in a situation in which the time was shortened between stimuli until the second spike did not appear. The usefulness of the stimulus of 10 x threshold was that it maintained the Q_{10} at a constant level.

FISHER inquired about size of nerve and electrode size in measuring threshold, for these would presumably affect the threshold as KEHL measured it. KEHL replied that the experimental procedure was strictly relative, that electrodes were of constant size, and that he always presumed the nerves used were of constant size.

BULLARD asked whether the curves were extrapolated or if he could actually measure a spike at low temperatures where there is no conduction. KEHL replied that he could not make such a measurement. He made the additional comment that temperatures as low as 4.5°C in the hibernating animal cause problems in measuring conduction velocity.

BARTHOLOMEW asked how velocities in hibernating mammals compared with what would be found in the lizard. KEHL

replied that he did not know. FISHER asked how the hibernating nerve compared with that of the frog. KEHL replied that the hibernating nerve had a temperature coefficient quite similar to that of the frog.

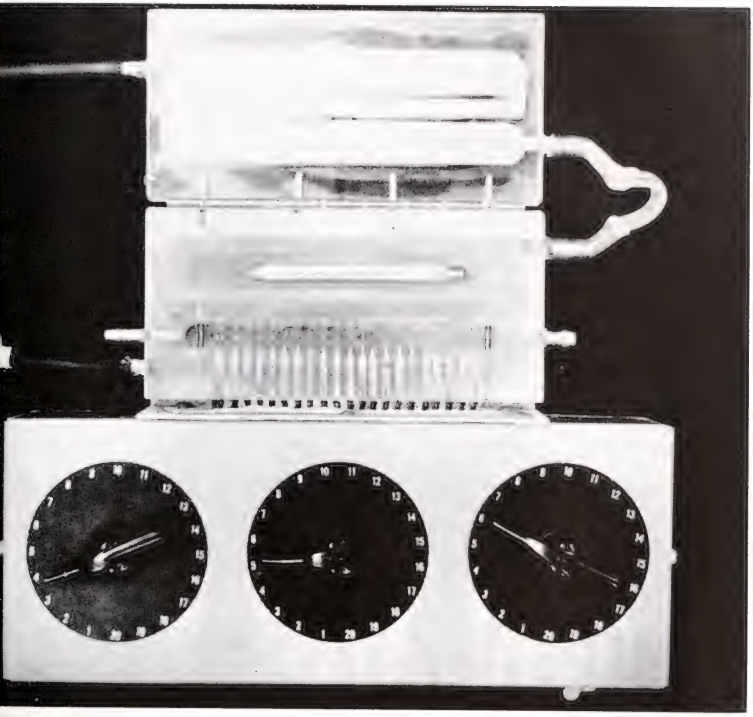
BISHOP remarked that the picture looked as though the phenomena observed could be obtained by raising the frog temperature coefficient up about half way or lowering the mammal nerve coefficient down about half way; therefore, the hibernating nerve seemed to stand between these two.

SCHÖNBAUM asked if anything was known of the biochemistry of nerves during hibernation as compared to non-hibernation. KEHL replied he was not qualified to answer. SCHÖNBAUM inquired whether anything was known about the effect of insulin or other hormones on nervous activity. KEHL remarked that his data were not in agreement with those of SUOMALAINEN, whose paper was presented earlier. He felt the differences may be clarified by doing hormone titers.

SOUTH noted that he would like to get more detail from KEHL on the design of his experiments: how many animals comprised a group at one temperature, whether he used mean values obtained from different nerves for his data. KEHL replied that in summer animals he did use mean values for ten samples, that the data agreed with P. O. Chatfield *et al.* (Am. J. Physiol., **155**:179, 1948). In the case of winter animals, a total of 30 animals was used; all nerves were run through a series of temperatures. SOUTH noted further that he had found that nerves do recover from a sojourn at a given temperature but they behave differently. He also stated that he used different procedures in threshold calculations. He asked whether voltage or milliamperage was being recorded, and whether it was monitored. KEHL replied that they used a single duration in measuring threshold, and used several times threshold at 0.1 msec. with a Grass stimulator which had been recalibrated. SOUTH suggested they use another beam on an oscilloscope as a monitor.

FISHER then asked what parameters were really measured. He remarked that if the duration of the stimuli were long enough and constant in length, the differences noted might conceivably be related to accommodation. KEHL replied that the stimulus duration was constant. FISHER then said that the variation in threshold might have to do solely with the size of the nerve.

KEHL replied that a single nerve was used in each case, hence size could not be a factor. FISHER remarked that variation in the water and salt content and hence the resistivity might also affect the threshold as measured, so that a "true" variation in excitability might not be involved at all. KEHL remarked that the change in temperature coefficients seen in peripheral nerves with the advent of hibernation seems to be associated with a decrease in hypophyseal secretory activity.



PLATE

Nerve Chamber. The stimulating electrodes are at the extreme left.

XXI

TISSUE RESPIRATION AND HIBERNATION IN THE THIRTEEN-LINED GROUND SQUIRREL, *SPERMOPHILUS TRIDECEMLINEATUS*[1]

By Marion P. Meyer and Peter Morrison
Departments of Zoology and Physiology
University of Wisconsin
Madison, Wisconsin

Animals which enter hibernation demonstrate a considerable decrease in metabolism along with a profound drop in body temperature. The total animal metabolism is, of course, a reflection of the respiration levels of the constituent tissue, and Martin and Fuhrman (1955) have shown that the summation of measured tissue respiration levels can give values reasonably close to observed basal metabolic rates. The relationship of the basal or intrinsic tissue respiration to the total animal metabolism is not completely understood, however, and the question arises as to whether the low metabolic rate observed during hibernation can be accounted for simply in terms of the temperature coefficients of the constituent tissues. In order to answer this question, the respiration levels and temperature coefficients of the tissues must be examined. The comparison of the hibernating animal in these respects with the non-hibernating animal of the same species and with mammals which do not hibernate will provide evidence as to possible intrinsic metabolic adjustments or adaptations.

In 1941, Hook and Barron, in their study of the role of brown fat in hibernation, measured the O_2 consumption of brown fat and kidney tissue from ground squirrels. In substrate-free media, the levels of respiration were the same in the hibernating and non-hibernating animal. The inclusion of various substrates considerably augmented the respiration level of kidney at 38° but not at 8°C and appeared to increase kidney respiration in the active animal slightly more than in the hibernating one. The Q_{10} of kidney was 1.9, and of brown fat, 1.4, when substrate

[1] Our program of studies on hibernation receives continuing support from the Wisconsin Alumni Research Foundation. This study was begun under USPH grant no. H-2095, assisted by the Wisconsin Heart Association and completed with aid from the American Cancer Society (Inst. grant to U. W.).

was not added; with succinate, the Q_{10} for kidney was 2.6, and for brown fat, 1.7.

Kayser (1954a,b) measured the O_2 consumption of kidney slices from hibernating and non-hibernating hamsters and white rats at a series of temperatures from 5° to 38° in a medium containing glucose. The data gave Q_{10} values of 2.3 for the white rat and 1.9 for both the hibernating and non-hibernating hamster. The hibernating hamsters, however, showed a uniformly lower oxygen consumption throughout the temperature range. Fuhrman and Field (1942) found a Q_{10} of 2.2 in the white rat. Thus, in the kidney of the hamster and ground squirrel, the temperature coefficient appears to be the same for the active and hibernating animals although the coefficient for the hamster seems less than for the white rat. The hibernating hamster showed a uniformly lower respiratory level than the active animal.

South (1958) published the results of oxygen consumption measurements at a series of temperatures from 5° to 43°C on heart ventricle and cerebral cortex of the white rat, active and hibernating hamster, and torpid bat. The temperature coefficients were not constant over this range for heart slices. Values were highest for the white rat, lower for the active hamster, and still lower for the hibernating hamster and torpid bat. On the other hand, values for oxygen consumption of brain slices indicated a Q_{10} of about 1.8 to 1.9 for all the four groups with a slightly lower level of metabolism in the hibernating hamster as compared to the active animal. Field *et al.* (1944) found a higher Q_{10} of 2.1 for cerebral cortex in a large series of white rats. Weiss (1954) gave a value of 2.2 for rat brain. Thus, while the brain showed no adaptation in the temperature coefficient, the heart of the hibernating hamster and bat appears to have a lower temperature coefficient resulting in a higher metabolic rate at the lower temperatures.

The bat and the hamster have been the principal hibernators studied in regard to tissue respiration. Bats hold a unique position in regard to hibernation in that they fall into a torpid state daily upon the cessation of activity, in contrast to the seasonal hibernation of other species. The hamster, on the other hand, might be termed a "permissive" hibernator since it stores food and may withstand low environmental temperatures for long periods of time without lowering the body temperature. Until now no extensive respiration measurements have been made upon any "obligate" hibernator which must almost always enter

hibernation in the fall and winter season. Accordingly, the present study of tissue respiration in the thirteen-lined ground squirrel was undertaken. And since each of the various body tissues thus far examined seems to present its own characteristic metabolic relationship to temperature, it was deemed desirable to examine a variety of individual tissues. By this means also we are able to arrive at an over-all metabolic picture of the influence of hibernation on tissue metabolism.

Materials and Methods

Thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*) were captured during the summer in the vicinity of Madison, Wisconsin. They were kept in small cages with access to water and rat ration and with supplements of greens and fruits. In the fall, a group of these animals were placed in individual cages in a hibernaculum—a bank of units individually isolated against light and noise and maintained at a temperature of 6°C by the circulation of refrigerated water through hollow "cold-walls." Each animal was supplied with cotton bedding material, water, and rat pellets. The period of hibernation was defined as the number of days since the last food or water was taken. This will represent a maximum period of hibernation, since the animal may have wakened from hibernation during the interval but not eaten. At this time it is not known which of these intervals is more significant. It is even possible that the total hibernation time during the season may be of greater importance.

Animals were removed from their cages, and rectal body temperature taken immediately after weighing and decapitation. The hibernating animals were dissected rapidly in a 5°C room. The organs were removed, weighed and cut into small pieces. They were then placed on filter paper moistened with inorganic Krebs medium in covered petri dishes which were kept on ice. The standard Warburg procedure for measuring the oxygen consumption of tissue slices was followed. Krebs medium III with phosphate buffer and glucose, pyruvate, glutamate and fumarate substrates and 1 per cent albumin was used to provide optimum conditions *in vitro*.

Measurements were carried out at 37.5° and 15°C on liver, kidney (cortex), spleen, lung, brain (cerebral cortex), diaphragm, heart (ventricle), and skeletal muscle (limb) of hibernating and active thirteen-lined ground squirrels. A few measurements were also carried out at 5°C. Also, several animals in intermediate

states of hibernation were studied. Two animals were cooled in an ice bath until their rectal body temperature dropped to 23° before decapitation. Another two animals were kept in the hibernaculum at 6° for several weeks after they had come out of hibernation in the spring. Two hibernating animals were allowed to warm spontaneously to 20°C before decapitation.

Results

The percentage of body weight which each organ represents is presented in Table I. These values are not given on a basis of fat-free body weight since, in most cases, the total fat contribution was not determined. There is considerable variability owing to the large weight changes which can occur with changes in fat deposit in these animals. The large standard deviations indicate this and make comparisons between the two groups difficult. However, the enlargement of the spleen known to occur during hibernation is apparent (Mann and Drips, 1917; Lidicker and Davis, 1955).

TABLE I

Organ Weights in *Spermophilus tridecemlineatus* as Percentage of Body Weight ± Standard Deviations [a]

Organ	Active [b]	Dormant [c]
Liver	4.20 ± 1.03	4.49 ± 0.93
Kidney	0.85 ± 0.25	0.90 ± 0.22
Spleen	0.19 ± 0.16	0.29 ± 0.15
Lung	0.73 ± 0.20	0.95 ± 0.25
Brain	1.58 ± 0.45	1.99 ± 0.43
Diaphragm	0.44 ± 0.07	0.45 ± 0.09
Heart	0.53 ± 0.35	0.63 ± 0.15
Stomach	0.96 ± 0.24	0.98 ± 0.19
Adrenals	0.016 ± 0.005	0.014 ± 0.005
Brown Fat	(0.32)	(1.68)
White Fat	(24.)	(13)
Skin	(14)	(15)
Sk. Muscle		(38)

[a] Parenthesized values for only a few animals.

[b] 23 non-hibernating animals ranging from 102-234 gm and averaging 155 gm.

[c] 20 hibernating animals ranging from 98-203 gm and averaging 127 gm.

A summary of the results of oxygen consumption measurements is presented in Tables II and III. The value at 37.5° for the kidney cortex of active ground squirrels, 30.8, approximates

TABLE II

Oxygen Consumption of Tissue Slices from Non-hibernating *Spermophilus tridecemlineatus.*

cc oxygen per dry gram hour (QO_2)

									$T_B = 23°C^a$ $T_A = 6°C^b$			
	5°C		15°C			37.5°C			37.5°C		15°C	
Tissue	No.	Mean	No.	Mean	S.D.	No.	Mean	S.D.	No.	Mean	No.	Mean
Liver	3	2.1	5	1.7	.4	14	4.6	1.3	2	5.9	2	2.6
Kidney	3	2.4	5	5.1	.3	14	30.8	5.3	2	23.1	2	5.9
Spleen	1	0.57	2	0.93	.1	10	8.6	1.2	1	8.0	1	1.3
Lung	2	0.35	3	0.76	.02	7	4.8	0.8	2	5.1	1	0.95
Brain	3	0.89	5	2.3	.3	14	12.5	2.3	2	14.1	2	2.5
Diaphragm	1	0.86	2	1.9	.4	10	2.6	0.8	1	3.6	1	2.4
Sk. Muscle	2	1.0	3	1.6	.2	6	2.8	0.6	—	—	1	1.6
Heart	3	2.3	5	3.6	.9	6	4.0	0.6	2	7.4	2	6.4

[a] Active animal cooled in ice water to 23°C body temperature.

[b] Active animal kept at 6°C following termination of hibernation.

TABLE III

Oxygen Consumption of Tissue Slices from Hibernating *Spermophilus tridecemlineatus.*

cc oxygen per dry gram hour (QO_2)

										$T_B = 20°C$a	
	5°C		15°C			37.5°C			15°C		37.5°C
Tissue	No.	Mean	No.	Mean	S.D.	No.	Mean	S.D.	No.	Mean	No.	Mean
Liver	3	1.9	13	2.2	0.5	7[b]	7.5	2.2	1	3.1	2	3.8
						8[c]	4.8	1.5				
Kidney	1	1.5	11	4.7	0.6	10	28.7	3.4	1	4.3	2	28.5
Spleen	1	—	3	0.82	0.2	5	8.6	1.6	-		-	-
Lung	1	0.22	7	0.72	0.2	7	5.3	0.9	—	—	1	5.3
Brain	1	0.79	11	2.4	0.5	10	14.7	2.8	1	2.6	2	13.9
Diaphragm	1	0.89	7	2.0	0.4	8	5.8	1.5	1	4.1	1	5.5
Sk. Muscle	1	—	4	1.5	0.1	4	4.4	0.6	1	2.4	2	4.8
Heart	3	2.4	13	4.4	1.2	6	6.8	2.4	1	5.8	2	7.4

[a] Hibernating animal warmed to 20°C body temperature.

[b] Hibernating less than 4 days.

[c] Hibernating more than 4 days.

the value of 27 cc/dry gm hr (QO_2) found by Hook and Barron (1941) who used just pyruvate as substrate. The metabolic levels for the tissues of the active thirteen-lined ground squirrel appear to be comparable to the levels found in the white rat, except in the case of muscle tissues. Our measurements using the same medium gave values of 5.5 for liver and 25.5 for kidney in the white rat, comparable to 4.6 in liver and 30.8 cc/dry gm hr in the kidney of the ground squirrel. On the other hand, rat diaphragm had a value of 6.4 as compared to 2.6 cc/dry gm hr in the active ground squirrel. Krebs (1950), using this medium, found values three times higher for liver and 50 per cent higher for kidney of the rat. Most investigators have used media containing only glucose for substrate and have found values slightly lower than those reported here, except in kidney for which literature values are about one half (Fuhrman and Field, 1942; Weiss, 1954). The difficulties in comparing tissue respiration values from various investigations are well known.

The muscle tissues measured in the ground squirrel seemed to have a lower respiration rate relative to other tissues than in a non-hibernator, the white rat. It should be noted that in the ground squirrel the respiration rate of muscle declined during the measuring period. Thus the initial rate of respiration would be higher than the mean value found for the hour of measurement. Extrapolations of the steady decline back to the time the flasks were placed in the constant temperature bath have been made. The decline was linear in the case of diaphragm and skeletal muscle and logarithmic for the cardiac tissue. The values derived by extrapolation, then, are considerably higher and equal to the normal rat values (which show less decline during measurement). However, errors due to changing rates of decline and extrapolation greatly increase the variability in duplicate measurements among the animals and make interpretation difficult.

At 15° none of the tissues studied from hibernating animals were significantly different from those from active animals, as is indicated in Table IV. The liver showed a slightly lower, and the kidney a slightly higher, respiration in the hibernating animal ($.1<P<.2$).

At 37.5°C the respiration of kidney, spleen, and lung from the hibernating animal was the same as from the active animal. The brain showed a significant, though small, increase of 20 per cent in hibernation ($P<.05$). The muscle tissues, on the other hand, increased their rate by 60 to 120 per cent in hibernation, so that

they were equal to that of the white rat at 37.5°. If the extrapolated values were considered, however, the respiration rate would be higher than for rat muscle. At 37.5° there was a transient increase in liver metabolism during the first few days of hibernation. Figure 1 presents values for liver in relation to the number of days since the animal last took food. During the first three days of hibernation the liver respiration was about 60 per cent higher than in the active animal. It then dropped back to the non-hibernating level. At 15° there was a possible

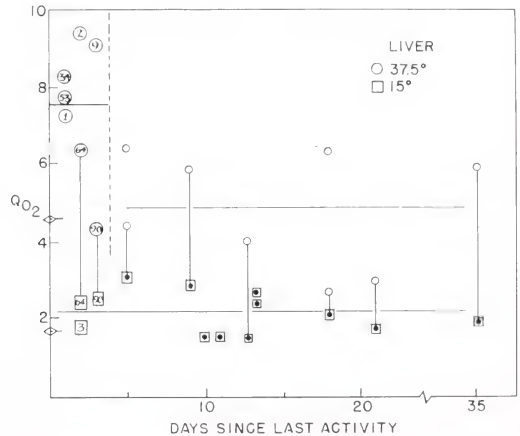


Fig. 1. Oxygen consumption of liver slices of the thirteen-lined ground squirrel in relation to time in hibernation. Numbers are days since *first* entering hibernation; vertical lines connect measurements on the same individual; horizontal lines indicate mean values; diamonds on ordinate show means for non-hibernating animals at 37.5° and 15°C.

slight increase in liver respiration throughout hibernation. The total number of days that the animal had been in hibernation during the season is given at each point in the early period. The transient rise in metabolism appears less evident in animals which have been hibernating for extensive periods before this last awakening and re-entry.

Table IV allows a comparison of tissue respiration in hibernating and non-hibernating animals in terms of the ratio of these values. The few measurements at 5° indicate a lower rate in

hibernating animals except in the case of the muscle tissues which did not change. More data is needed to verify this, however. At 15° the tissues studied showed the same respiratory rates in the active and hibernating animals. At 37.5°, however, the liver (during the first three days) and the muscle tissues demonstrate a considerable increase in respiration in the hibernating animal over the active one.

The values from two active animals which were force-cooled to 23° indicated an increase in the respiration of cardiac muscle to the hibernating level or higher, and a lesser increase in liver and diaphragm (Table II). The kidney apparently decreased

TABLE IV

Influence of Hibernation on Oxygen Consumption of Tissue Slices (H:N-H ratios)

	5°	15°	37.5°	37.5° ($T_B = 20°$)
Liver	0.91	1.3**	1.6* < 4 days 1.0 > 4 days	1.27 .81
Kidney	0.62	0.92**	0.93	1.2
Spleen	—	0.88	1.0	1.1
Lung	0.63	1.1	1.1	1.0
Brain	0.89	1.1	1.2*	1.0
Diaphragm	1.0	1.1	2.2*	1.6
Sk. Muscle	—	0.95	1.6*	—
Heart	1.0	1.2	1.7*	0.92

* $P < .05$
** $.05 < P < .2$.

in oxygen consumption. These changes may well be the result of immediate hormonal variations under this stressful situation. It is of interest that the muscle tissues and liver were again the organs which showed increased metabolic activity.

Another two animals were maintained in the hibernaculum at an ambient temperature of 5° for several weeks after their natural awakening from hibernation in the spring. The values presented in Table II indicate increased metabolic levels in liver, diaphragm and most particularly heart when measured at 15°. Such changes at the lower temperature were not found in hibernation; again muscle and liver are the tissues indicating changes. Weiss (1957) has shown that these are the tissues which show

increased metabolism in white rats that have been cold-adapted. Thus, these preliminary experiments indicate that a hibernator when not in hibernation may utilize similar mechanisms for cold adaptation as non-hibernators.

The tissue respiration levels of two hibernating ground squirrels which were allowed to warm to 20° during arousal indicated an increase in the metabolism of muscle tissues and liver at 15°, as in cold adaptation (Table III). However, at 37.5° there appeared to be no changes. The results of these preliminary experiments involving only one or two animals are, of course, not definitive. However, they do show the adaptability of muscle and liver tissue as compared to the relatively unchanging metabolic levels of spleen, lung, brain, and kidney.

TABLE V

Temperature Coefficients for *Spermophilus tridecemlineatus:* Q_{10}

	Non-Hibernating		Hibernating	
Organ	5°-15°	15°-37.5°	5°-15°	15°-37.5°
Liver	0.81	1.56	4.16	2.37 < 4 days 1.53 > 4 days
Kidney	2.12	2.22	3.13	2.23
Spleen	1.63	2.70	——	2.84
Lung	2.17	2.29	3.73	2.42
Brain	2.60	2.12	3.04	2.24
Diaphragm	2.21	1.15	2.23	1.61
Sk. Muscle	1.60	1.28	——	1.62
Heart	1.56	1.05	1.83	1.22

Q_{10} values as determined between 5° and 15°, and 15° and 37.5° are presented in Table V. The Q_{10} values between 15° and 37.5° for liver, kidney, and brain in hibernating and active animals are in the range of expected values based upon white rat studies (Fuhrman and Field, 1942; Field *et al.*, 1944; Fuhrman and Field, 1945; Fuhrman *et al.*, 1950; Kayser, 1954a; Weiss, 1954). South (1958) found also that there were no differences in temperature coefficients of brain from the white rat, torpid bat, and hibernating and active hamsters. No reports were found in the literature on temperature coefficients of metabolism in lung and spleen. The Q_{10} of 2.7 for spleen is appreciably higher than the values for other tissues. It would be

of interest to know whether this high Q_{10} in the ground squirrel is typical also of splenic tissue from non-hibernating mammals.

The Q_{10} values in the 5° to 15° range are based upon only 1 to 3 measurements at 5° and therefore should be considered as preliminary indications of a higher temperature coefficient at the lower temperatures in the liver, kidney, lung, and brain of the hibernating animal. In the active animal the Q_{10} appears to be lower in the liver and spleen, about the same in kidney and lung, and higher in the brain at this lower temperature interval. Throughout this group of tissues the temperature coefficients of the hibernating animals were higher than the active. South (1958) found in brain a higher temperature coefficient for both the hibernating and non-hibernating hamster between 5° and 10°, as was found here for the ground squirrel. However, the active hamster showed the greater increase, whereas in the ground squirrel the hibernating animal increased more.

At the higher temperature interval, the active animals showed very low Q_{10} values of 1.0 to 1.3 for muscle tissue, as might be expected from their low respiration values at 37.5°. That these values may be below the true respiration level due to declining rates during measurement has already been indicated. At 15° also there is a decline in respiration in the muscle tissues. However, the relative rate of decline, particularly in the heart, is less than at 37.5° and thus the Q_{10} would actually be a little greater than expressed here. Even using these extrapolated values, the Q_{10} for heart is only 1.2. Somewhat higher Q_{10} values of 1.7 (Weiss, 1954) for diaphragm, and 1.5 (Weiss, 1954) and 1.7 (South, 1958) for heart have been found in the white rat.

The respiration of diaphragm and limb muscle of the hibernating ground squirrel gave Q_{10} values of 1.6 (1.8 extrapolated) which approximate the 1.7 for the white rat and are higher than the non-hibernating values. The Q_{10} of 1.2 for cardiac muscle is raised to 1.6 by the use of extrapolated values. This value falls between the 1.5 found by Weiss and the 1.7 by South for white rats and is higher than South's extrapolated value of 1.36 for the hibernating hamster. The Q_{10} for muscle then is about the same in the hibernating ground squirrel as in the white rat, while in the non-hibernating animal it is lower. South found the Q_{10} of heart from the active hamster to be lower than that of the rat. However, the heart Q_{10} of the hibernating animal was still lower.

TABLE VI

Summary of the Alterations in Tissue Metabolism Observed in *Spermophilus tridecemlineatus*

	Spermophile vs. White Rat			Spermophile								White Rat[a]
				Hibernating vs. Non-hibernating					Waking vs. Hibernating		Force Cooled vs. Non-hibernating	Cold Adapted vs. Non-hibernating	Cold Adapted vs. Normal
						Q_{10}							
	15°	37.5°	Q_{10}	5°	15°	37.5°	5°-15°	15°-37.5°	15°	37.5°	37.5°	15°	37°
Muscle	0	—	—	(0)	0	+	(0)	+	(+)	(0)	(+)	(+)	+
Liver	0	0	0	(0)	0	+ 0	(+)	+ 0	(+)	(0)	(+)	(+)	+
Others	0	0	0	(−)	0	0[b]	(+)	0	(0)	(0)	(0)	(0)	0

[a] Weiss (1957).
[b] Brain (+20% ($P < .05$)).

Discussion

Table VI presents a summary of preceding results in order that differences and similarities among these groups and these conditions may be kept clearly in mind. The tissues studied fell into three categories in regard to metabolic changes under various conditions.

First, the kidney, spleen, lung, and brain of the ground squirrel showed respiration levels and Q_{10} values equivalent to those of the white rat; moreover, no alterations occurred during hibernation except for a possible increased Q_{10} at low temperatures. Accordingly, these tissues appear to be unmodified in respect to hibernation function.

Second, liver, which differs from all other tissues, shows a transient increase in metabolism in the hibernating animal at 37.5°, and possibly a slight increase at 15° throughout hibernation. In the early hibernation period Zimny and Tyrone (1957) found a transient elevation of glycogen levels which then dropped to non-hibernating levels after 3 days. Thus it appears that there are metabolic adjustments in the liver during the first few days of hibernation. The arousing animal (at 15°), the force-cooled active animal (at 37.5°), and the cold-adapted animal (at 15°) all showed increased liver respiration.

The third group, muscle tissues (heart, diaphragm, and skeletal muscle), demonstrated metabolic rates in the non-hibernating animal below the expected values for the white rat at 37.5° but not at 15° or 5°. The values for hibernating animals approached those for the normal rat at all temperatures. Thus the low Q_{10} in the active animal increased during hibernation to the level of the white rat.

The first question which comes to mind in interpreting these results on tissue respiration is their relationship to the changing metabolic rate of the intact animal: Can the low metabolic rate of the animal during hibernation be accounted for in terms of the temperature coefficients of the constituent tissues? The data of Hock (1951) on metabolism of the little brown bat (*Myotis lucifugus*) yield a single Q_{10} of 3.7 between 2.0 and 37° when minimum values at each temperature are used. This uniform temperature coefficient over a wide range suggests that the intact animal may be treated as a single system in regard to its response to temperature. The bat lends itself to such analysis since it may assume a hibernating "posture" at any point over the whole temperature range. Data over more limited ranges

are available for other hibernators, but temperature coefficients may be calculated from the basal metabolic rate and values at 5-10°C. Thus from the data of Kayser (1940) a Q_{10} of 3.7-3.9 may be calculated for the spermophile, *S. citellus*, and the dormouse, *Glis glis*. The temperature coefficient of a North American spermophile, *S. undulatus*, was 3.55 (Morrison, 1960).

It is apparent that the Q_{10}'s of all tissues are much lower than this. The muscle tissues which contribute about one-quarter of the total metabolism have a Q_{10} of 1.3. The overall average Q_{10} (weighted) then is less than 2.0 in the 15° to 37.5° interval. The greater temperature coefficient in the 5° to 15° interval, however, would bring the average Q_{10} to about 2.3. This is still considerably lower than the values for the total animal metabolism (by a factor of 3.4 at 5°). Extrinsic controls or influences are thus necessary for the regulation of tissue metabolism at the reduced levels observed in hibernating animals.

Upon first approaching the study of hibernation, one tends to look for specializations in the animal during hibernation since this is considered the unusual condition. It may be, however, that the most difficult problems are met during the transitional periods of entrance into and arousal from the hibernating state. And the low Q_{10} of muscle tissues in the non-hibernating ground squirrel might be an adaptation to maintain function in the heart and diaphragm during entrance into hibernation. Thus, in cooling to 5°, cardiac metabolism only dropped to about 60 per cent of the value at 37°C.

On the other hand, the modification which takes place during hibernation and which results in a greater temperature coefficient, but in no change in the 15° level, would not influence hibernation itself. It would, however, be advantageous in facilitating the awakening process during which there is a tremendous demand for energy necessary for the rapid warming of the animal. The cardiac and skeletal muscle are considered of major importance in this rewarming process (Lyman and Chatfield, 1955). However, the Q_{10} of metabolism during arousal is still much higher. Thus, although the Q_{10} of muscle metabolism increases during hibernation, it is still completely inadequate to account for the high temperature coefficient of the whole animal on awakening. Obviously, extrinsic hormonal and/or nervous regulation are superimposed upon the basal tissue respiration in order to achieve this maximal heat production. The muscle metabolism of animals partially aroused appeared to increase

over the hibernating value at 15°. During arousal from hibernation the animal may be considered to be under stress, as during force cooling and cold adaptation. And in each of these cases the muscle metabolism showed increased respiration rates although, of course, insufficient to account for the rise in the intact animal metabolism.

Summary

The respiratory rate and Q_{10} values of kidney, spleen, lung, and brain from the thirteen-lined ground squirrel showed no evidence of adaptation either in the active or in the hibernating animal, except for a possible decrease at 5° in hibernation. The liver, on the other hand, demonstrated a transient increase in metabolism early in hibernation and an increase in several stressful situations (arousal, force-cooling, cold adaptation). The muscle tissues all showed reduced temperature coefficients in the non-hibernating animal. These rose to standard values (white rat) during hibernation. Moreover, the muscle tissues, and particularly the heart, showed increased metabolic rates during the stress situations. Thus, the muscle tissues show metabolic adaptations appropriate to hibernation: a reduced temperature coefficient in the active animal which might allow successful functioning of the heart upon entering hibernation, and an increased coefficient during hibernation which might facilitate the awakening process. These intrinsic temperature coefficients do not in themselves account for the pattern of changing metabolism in the intact animal, but must interact with extrinsic nervous or hormonal factors.

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DISCUSSION FOLLOWING MEYER'S PAPER

ZIMNY commented that in studying liver metabolism one should not worry about large variations in results. For example, if one looks at glycogen levels in the liver, one finds this organ "will do whatever it can for other organs." Studying the compounds of intermediary metabolism showed that the liver aided the heart and skeletal musculature, and that this caused great variation in the levels of compounds in the liver. MEYER assented.

BULLARD asked whether the metabolic rates taken on individual tissues as related to weights for the organs from which those tissues were obtained equalled in sum the metabolic rate values for the whole animal. MEYER replied that this had been done by A. W. Martin and F. A. Fuhrman (Physiol. Zool., **28**:18, 1955), who found that the summed respiration rates of the organs and tissues for the mouse and the dog were reasonably close to the observed metabolic rate of the intact animal. However, the level of the metabolic rate of tissues *in vitro* is in part a function of the system used in measuring it, so much so that the many factors involved make it difficult to know the actual rate comparable to that of the tissue *in situ*. In tissue studies, the best one can realize is a comparison of metabolic rates and this comparative picture is what is obtained here.

XXII

THE INTERNAL ENVIRONMENT DURING HIBERNATION

By Marvin L. Riedesel
Department of Occupational Health
Graduate School of Public Health
University of Pittsburgh, Pittsburgh, Penn.

Lowered body temperature and reduced metabolic rate for an extended period of time are included in the simplest description of hibernation. Recently, we have learned to expect numerous biochemical changes during hibernation. Biological processes are recognized as being subject to modification by these three factors: temperature, time and chemical environment. Before considering the biochemical changes which occur with hibernation, let us consider briefly some examples of the interdependence of these factors. The interdependence of time and chemical environment in biological processes is demonstrated by the catalytic action of enzymes and ions. The interaction of temperature and chemical environment is illustrated by Bachrach's studies (Bachrach, 1946). He observed a drop of 17°C in the optimum temperature for contraction of the snail (*Helix aspera* Mull) heart when Kreb's solution was replaced by isotonic magnesium chloride. The optimum temperature for contraction in isotonic potassium and sodium chloride was intermediate. Other demonstrations of the interdependency of chemical environment and temperature were made by Conway and Geoghegan (1955) and by Adolph and Richmond (1956). They reported a gain in weight when tissues were suspended in cold isotonic solutions. Adolph and Richmond have suggested a redefinition of isotonicity in terms of temperature and time. Interdependence of temperature and time is demonstrated by the effect of time upon the optimum temperature of enzymes and enzyme systems (Spector, 1956; Dixon and Webb, 1958). The importance of time in defining tolerance limits of animals to heat and cold is another example of the interdependence of time and temperature. It appears that a study of biological processes during hibernation must consider the interdependence of temperature, time and chemical environment.

Serum Electrolyte Levels During Hibernation

The relation of the magnesium ion to temperature regulation has given particular interest to the elevation of serum magnesium during hibernation. Three types of studies other than hibernation have related the magnesium ion to heat loss. Parenteral injection of magnesium has been demonstrated to facilitate hypothermia (Schutz, 1916; Heagy and Burton, 1948; Hall *et al.*, 1951) and elevated serum magnesium has been reported to be produced with hypothermia in a number of animals (Steadman *et al.*, 1943; Platner, 1950; Platner and Hosko, 1953). The antipyretic action of magnesium has been described in experimental animals and in human beings (Barbour and Winter, 1928; Sollman, 1957).

TABLE 1

Reports on Serum Magnesium during Hibernation

Common Name	Scientific Name	Increase over Controls	Investigator
Thirteen-lined ground squirrel	*Citellus tridecemlineatus*	65%	Riedesel and Folk (1957)
Woodchuck	*Marmota monax*	63%	McBirnie *et al.* (1953)
Golden hamster	*Mesocricetus auratus*	25%	Riedesel and Folk (1957)
Little brown bat	*Myotis lucifugus*	62%	Riedesel and Folk (1957)
Big brown bat	*Eptesicus serotinus*	53%	Riedesel (1957)
Hedgehog	*Erinaceus europaeus*	92%	Suomalainen (1939)
Hedgehog	*Erinaceus europaeus*	none	Björck *et al.* (1956)

Elevation of the serum magnesium appears to be a characteristic of hibernation. Reports of elevated serum magnesium have been made on a large sample of the hibernators by several independent laboratories (Table I). The extent of the increase in serum magnesium is dependent upon the species of the hibernator. Some of the details of the change in serum magnesium were determined in studies on the little brown bat. There was no significant increase in the magnesium when esophageal temperature had lowered to 17°C, but an elevation of serum magnesium was observed when the esophageal temperature had dropped to 13°C (Fig. 1). The elevation of serum magnesium appears to

be dependent upon cooling of cells. Bats raised their body temperature to the semiactive level of 18°C in four to eight minutes without altering the hibernation level of serum magnesium (Fig. 2). The magnesium level appears to be independent of temperature during arousal from hibernation. Then, when the body temperature was raised further by exposure in a warm room for one hour, the serum magnesium was back to the basal level of

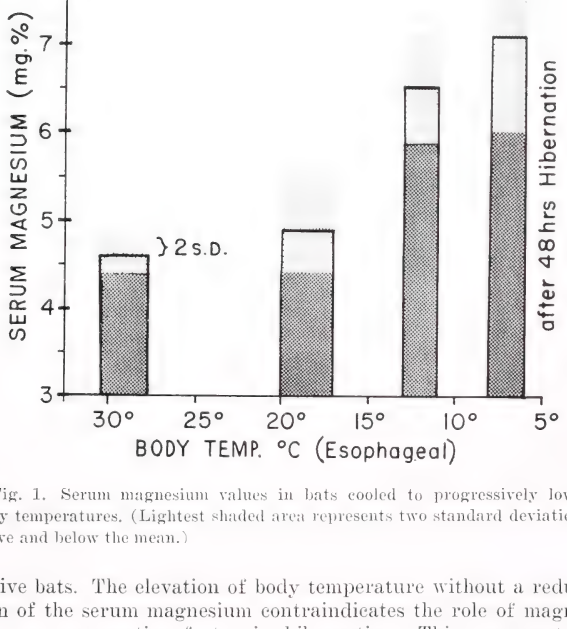

Fig. 1. Serum magnesium values in bats cooled to progressively lower body temperatures. (Lightest shaded area represents two standard deviations above and below the mean.)

active bats. The elevation of body temperature without a reduction of the serum magnesium contraindicates the role of magnesium as a causative factor in hibernation. This argument is weakened by the fact that awakening from hibernation appears to be a very different process from "going into" this state.

Reports on serum electrolytes other than magnesium describe primarily homeostasis of potassium, sodium and calcium during hibernation. There are some exceptions. Depending upon the

species studied, the serum potassium remains near control levels or increases during hibernation. An 82 per cent increase in the serum level of potassium during hibernation of the woodchuck has been reported (McBirnie *et al.*, 1953) (Table II). A consistent increase in serum potassium has been observed with active hamsters, ground squirrels and bats in a cold environment (Riedesel and Folk, 1958). The cause and effect relationship of the changes in serum potassium are not apparent, but

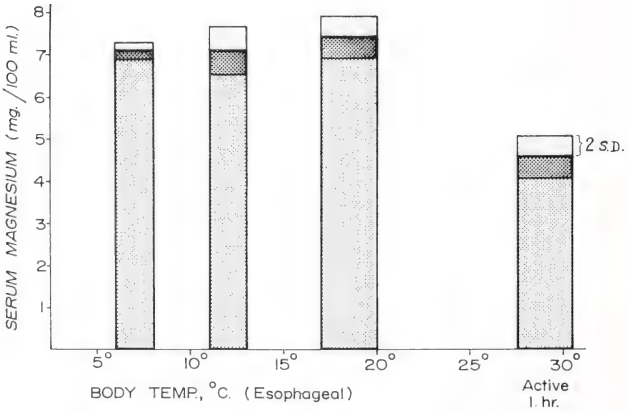

Fig. 2. Serum magnesium levels during arousal from hibernation.

the changes in serum potassium during hibernation may be effected by adrenal cortical activity or utilization of glycogen. Fenn and Asano (1956) have reported an increase in intracellular potassium with deposition of glycogen. Thus, the increase of serum potassium may result from the reduction in glycogen stores which occurs during hibernation (Zimny, 1956). The values of serum sodium during hibernation of the hedgehog were similar to those found in active animals (Suomalainen, 1939, 1953; Björck *et al.*, 1956). Reports of serum calcium concentration during hibernation are controversial. No change has been reported with hibernation of the ground squirrel, hamster and hedgehog, whereas hypocalcemia has been observed with hibernation of the little brown bat and European marmot. An 88

per cent increase in the serum calcium was reported with hibernation of the European marmot (Ferdmann and Feinschmidt, 1932). A more detailed study of the little brown bat has given rise to interesting speculation. The data in Figure 3 suggest that the serum levels observed during hibernation may vary with the body temperature and/or depth of hibernation. The lowering of serum calcium with reduced body temperature does not appear to be large, but it is consistent. The lowering of serum

TABLE II

Reports on Serum Electrolytes during Hibernation

Animal	Electrolyte Concentrations as Compared to Controls			Investigator
	Potassium	Calcium	Sodium	
Woodchuck	increased			McBirnie *et al.* (1953)
European marmot		decreased		Adler (1926)
	decreased	increased		Ferdmann and Feinschmidt (1932)
Thirteen-lined ground squirrel	no change	no change		Riedesel and Folk (1958)
Hamster	no change	no change		Riedesel and Folk (1958)
Little brown bat	increased*	decreased**		Riedesel and Folk (1958)
Big brown bat	no change			Riedesel and Folk (1958)
Hedgehog	decreased	no change	no change	Suomalainen (1939) (1953)
	no change	no change	no change	Biörck *et al.* (1956)

* The increase was consistent but not statistically significant.

** The serum calcium appears to vary with depth of hibernation.

calcium with hibernation of the little brown bat receives further support from the observation of a 54 per cent reduction in serum calcium in bats which had hibernated for ten weeks and a 34 per cent decrease after nine weeks of hibernation (Riedesel, 1957). The low and apparently fluctuating calcium levels with extended hibernation cannot be explained on the basis of the data available. The calcium level may cycle during long-term hibernation, and the data cited here may have been taken at times when the calcium level happened to be at a low ebb of the cycle. Such a cycle may account for the contradictory reports on the calcium content of the serum during hibernation which appear

in Table II. It may be that during hibernation the circulation to calcium stores and/or kidney function is not consistently adequate to meet the tissue demands for calcium, and at the same time maintain a constant serum calcium level.

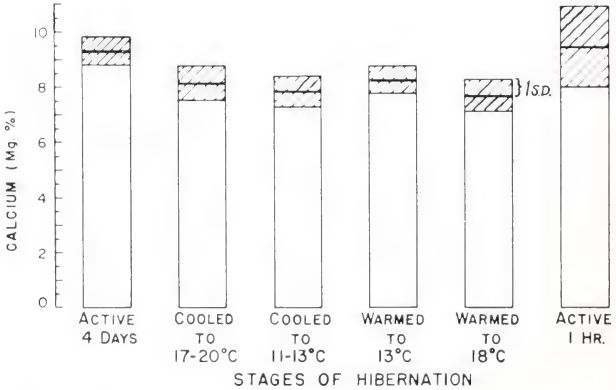

Fig. 3. Serum calcium measurements of *Myotis lucifugus* in stages of hibernation. (Shaded area represents one standard deviation above and below the mean.)

Body Fluids

Few measurements of body fluid have been made during hibernation. The susceptibility of the hibernating animal to manipulation limits the success of such measurements. Small decreases in plasma volume are indicated by blood volume and total serum protein measurements. The increase in blood volume/body weight during hibernation of the hamster was largely explained by loss of body weight (Lyman *et al.*, 1957). An increase in serum concentration is indicated by the 21 per cent increase in total serum proteins during hibernation of the hamster (South and Jeffay, 1958). Actually, information regarding blood and plasma volume is limited to hematological observations. The hematological studies show inconsistent results (Table III). The data on the golden hamster and European marmot indicate concentration of the blood, if not the plasma. Cyclic

shifts of body water may account for the controversial hematological data. The electrolyte data cited earlier indicate homeostasis with regard to body fluid compartments since magnesium, potassium and calcium concentrations change independently of each other; for instance, if the elevation of serum magnesium during hibernation were due to a decrease in plasma water, one would expect similar increases in serum potassium, calcium and sodium values. The urine of hibernating ground squirrels has been reported to be of very small volume and dilution

TABLE III

Indices of Hemoconcentration during Hibernation

Animal	Criteria	Change with Hibernation	Hemo-concentration	Investigator
Thirteen-lined ground squirrel	Erythrocyte count	decreased	−	Stuckey and Coco (1942)
	Erythrocyte count	increased	+	Svihla and Bowman (1953)
	Hematocrit	sl. increase	+	Svihla and Bowman (1953)
	Hematocrit	no change	0	Riedesel (1957)
	Serum sp. gr.	no change	0	Riedesel (1957)
Woodchuck	Erythrocyte count	decreased	−	Rasmussen (1916)
	Hematocrit	increased	+	McBirnie *et al.* (1953)
	Hemoglobin	no change	0	Rasmussen (1916)
	Blood sp. gr.	no change	0	Rasmussen (1916)
European marmot	Blood sp. gr.	increased	+	Dubois (1896)
	Erythrocyte count	increased	+	Dubois (1896)
	Water content of blood	decreased	+	Aeby (1875) Dubois (1896)
Hamster	Erythrocyte count	increased	+	Lyman *et al.* (1957)
	Hematocrit	increased	+	Lyman *et al.* (1957)
	Hematocrit	increased	+	Riedesel (1957)
	Hematocrit	increased	+	South and Jeffay (1958)
	Serum sp. gr.	no change	0	Riedesel (1957)
	Total Serum proteins	increased	+	South and Jeffay (1958)
Little brown bat	Hematocrit	no change	0	Riedesel (1957)
	Serum sp. gr.	no change	0	Riedesel (1957)
Big brown bat	Hematocrit	no change	0	Riedesel (1957)
	Serum sp. gr.	increased	+	Riedesel (1957)
Hedgehog	Hematocrit	increased	+	Biörck *et al.* (1956)
	Total serum proteins	no change	0	Biörck *et al.* (1956)

(Hong, 1958). Additional information on kidney function would facilitate an understanding of water balance during hibernation. No major shifts in body water during hibernation are apparent from existing data.

Other Changes in the Internal Environment

The subjects of pH, pO_2 and pCO_2 of the blood, energy sources, blood sugar and endocrines have been reviewed recently by Lyman and Chatfield (1955) and Kayser (1957). These data accumulated on hamsters, woodchucks and ground squirrels indicate that hibernating animals maintain oxygenation of the blood, pH and pCO_2 near the values of active animals. Data on the respiratory quotient and blood glucose levels indicate that fat is the principal source of energy during hibernation. The role of the endocrines as a causal factor in hibernation is questionable, but changes in endocrine activity may effect a pre-conditioning of the animal for entrance into hibernation. The facility of some animals to hibernate in the laboratory the year round contradicts the supposition that endocrines have a causative role in hibernation (Riedesel, 1957).

Theories on the Development of Hibernation

The elevation of serum magnesium and the relation of the magnesium ion to heat loss in other types of studies suggest magnesium as a causal factor in the development of hibernation. We have presented several theories on the development of the state of hibernation with reference to magnesium (Riedesel, 1957).

The "Independent-Influence Theory" implies that exposure to a cold environment independently alters the activity of the hypothalamus and also produces elevated serum magnesium. The serum magnesium level has no causative role in the decrease in body temperature. This theory receives support from the evidence that elevation of magnesium and lowered body temperature are separable. This was observed during arousal from hibernation when bats raised their body temperature to 20°C without lowering the serum magnesium.

The "Direct-Influence" theory simply means that cold exposure and the resulting cooling of cells directly produce a release of magnesium by the cells, and the resulting elevated level of serum magnesium influences the hypothalamus to increase heat loss. This theory receives support from two observations.

The first is that a high level of magnesium was observed before the temperatures of deep hibernation were reached. Thus, the elevated magnesium observed with 13°C esophageal temperature may facilitate the development of the lower temperatures. The second line of evidence in support of this theory is that intravenous and micro-injection of magnesium ion have been demonstrated to facilitate heat loss (Schutz, 1916; Heagy and Burton, 1948; Hall *et al.*, 1951).

The "Additive-Influence" theory assumes that exposure to the cold and elevated serum magnesium both influence the activity of the heat loss center and have an additive effect. The theory implies that cooling of tissues, endocrine and cerebral cortical activity and other factors initiate the lowering of body temperature and that the elevated serum magnesium facilitates the process by affecting the heat loss center. This theory receives support from the observation that the serum magnesium had not increased when the esophageal temperature had dropped to 17°C. This evidence indicates that a factor other than elevated magnesium is required for initiating the decrease in body temperature.

The author is of the opinion that the "Additive-Influence" theory is the most acceptable in view of the evidence cited. Elevation of the esophageal temperature to 20°C during the arousal from hibernation without reduction of the serum magnesium level represents the major evidence that contradicts this theory. This argument is weakened by the fact that awakening from hibernation appears to be a very different process from "going into" this state. Arousal from hibernation can be initiated by discrete external stimuli, such as handling. Internal stimuli from thirst, hunger or a full bladder are also undoubtedly involved in arousal from hibernation. No such stimuli have been related to the development of hibernation. Arousal from hibernation must be the result of neural stimulation of the hypothalamus and this stimulation must be strong enough to overcome the influence of magnesium on the heat loss center.

To restate the "Additive-Influence" theory, the development of hibernation may proceed in the following manner: Cold exposure and reduced activity produce a cooling of peripheral tissues, and a release of magnesium from cells to plasma occurs. This may start when the animal is asleep. The increase in serum magnesium affects the heat loss center so that body temperature and metabolism of the animals drop to hibernation levels.

Questions and Suggestions for Future Research

Questions and suggestions to be considered when planning research on hibernation include the following:

1. More information is needed regarding the interdependence of temperature, time and chemical environment in biological processes.

2. The chemical environment and temperature of *in vitro* studies should be similar to those described *in vivo*.

3. Are changes in intracellular electrolyte concentrations a universal response of cells to cooling?

4. Additional information is needed on water balance during hibernation; for instance, "What is the extent of the kidney activity during hibernation?"

5. Additional information is needed regarding the possible cyclic changes in serum calcium concentration during hibernation.

6. We need a better definition of hibernation in terms of species differences and stages of hibernation.

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DISCUSSION FOLLOWING RIEDESEL'S PAPER

WIMSATT observed that RIEDESEL'S serum measurements could reflect discontinuity in hibernation, and might also provide evidence for arousal. If so, one may be able to manipulate mechanisms in such a way as to eliminate arousal. He also gave further evidence for cycling of hibernation. In visiting bat caves every two weeks during three winters he observed that the clusters of bats moved, and banding studies showed movements of animals between caves in the middle of winter. Even in an artificial hibernaculum there are times when bats are active in the hibernating season. RIEDESEL thought this was true, and he felt that the cycle may partly reflect temperature changes. With respect to movements of bats in caves, FOLK (G. E. Folk, J. Mammal., **21**:306, 1940) was cited as having reported changes in positions of bats in winter, quite a bit of movement being characteristic.

ADOLPH then asked if SUOMALAINEN would give his present view of the role of magnesium in hibernation. SOUTH also asked if SUOMALAINEN held to his old viewpoint. SUOMALAINEN remarked that it is a difficult question, but that he was sure magnesium is a typical indicator of the hibernating state, whether in a primary or secondary role he could not say.

RIEDESEL remarked that the data cannot be interpreted readily to mean that magnesium is a factor in initiating hibernation, and that he did not intend to leave the impression that magnesium initiates hibernation.

POPOVIC stated that he felt bats were not well-chosen animals for this work since they differ considerably from ground squirrels and other hibernators. A hibernating ground squirrel and an artificially cooled ground squirrel would probably show a big physiological difference in respect to magnesium, in contrast to bats. Other striking contrasts are seen; for example, the

blood pressure is low in hibernation, whereas in the artificially cooled animal, it is 90 mm Hg. Heat production is 5 to 15 times higher in cooled than in hibernating animals. He believed that further experiments should be performed to see if the changes in magnesium should be attributed to hypothermia or to hibernation.

RIEDESEL replied that he had "heard rumblings as to whether the bat is a good example of a hibernator." He defended the bat as a hibernator, saying that he had spent 10-11 hours in a cold room waiting for bats to go into hibernation to make study possible. He checked each one individually every 15 minutes. They remained relatively active and kept their body temperature up. He checked body temperature until it lowered to 20°C, then drew blood and analyzed it. Sometimes body temperature rose 7-8°C in 15 minutes, but half an hour later it would be down again. Concerning changes in body temperature and magnesium, he believes that on the cellular level hypothermia and hibernation are quite similar. With respect to the whole ground squirrel put into hypothermia, he felt that differences would exist between this condition and hibernation at system, organ or tissue levels, but not at the cellular level.

SCHÖNBAUM said that an increase in magnesium in the hibernator's blood had to come from somewhere; did RIEDESEL have any idea as to which part of the organism gave up magnesium first — body tissues generally or specific organs, such as muscle? He also wondered what happens to the magnesium which has entered the serum. RIEDESEL said that he had no evidence on this, but his impression was that temperature affects the extracellular:intracellular magnesium ratio which at 37°C is 20:1. As peripheral cells (skin and muscle) are cooled, this ratio becomes smaller and the serum magnesium level increases. He believes that the first release of magnesium into the serum occurs in the peripheral tissues where the temperature is lower. In nature, bats in the temperate zone go in and out of hibernation at least once a day in the spring and fall, and since there is no evidence of magnesium deficiency, it is doubtful that extraordinary amounts of magnesium are excreted in the urine. He believes magnesium does not leave the body during hibernation, but returns from plasma to cells on arousal from hibernation.

KAYSER then stated that he believes there is a clear relationship between magnesium levels and hypothermia. One sees an

increase of serum magnesium in hibernation and in hypothermia. He believes magnesium increase in the serum is related to (is perhaps the consequence of) hypothermia. He said he was not convinced that magnesium had a causal effect on hibernation, but rather believed that elevated serum magnesium was a consequence of cold.

FOLK remarked that he felt the discussion was difficult because there was not time enough to go into methods. Relative to WIMSATT'S and POPOVIC'S comments, he said it should be emphasized that measurements were made after sacrificing animals in spontaneous hibernation.

BARTHOLOMEW, extending KAYSER'S remarks, pointed out that hibernation-hypothermia discussions are impeded by the chronic problem of causation and correlation. He would like to separate hypothermia and hibernation. By experience with a variety of forms, he had noted that hypothermia, *per se*, is not an essential causal factor for hibernation. He felt that peripheral cooling is a result rather than a cause. He asked that we release ourselves from dependency on the idea that cold exposure is a necessary prior condition to entering hibernation.

RIEDESEL stated that it is difficult to designate magnesium as a causative factor in initiating hibernation. However, it appears logical that the elevated serum magnesium (by its effect on the heat loss center) facilitates loss of body heat and the reduction of body temperature to levels characteristic of deep hibernation.

XXIII

A STUDY OF THE METABOLISM OF LIVER, DIAPHRAGM AND KIDNEY IN COLD-EXPOSED AND HIBERNATING HAMSTERS[1]

By Arliss Denyes and Joan Hassett
Biology Department
Queen's University
Kingston, Ontario, Canada

Of the many problems in hibernation two basic ones can be broadly defined: 1) the elucidation of the mechanisms for entrance into and arousal from hibernation, and 2) the nature of the intermediary metabolism of the tissues of hibernators when the animals are exposed to varying climatic conditions, when entering hibernation, in hibernation and when arousing. In regard to the second problem we are fortunate in having a wealth of literature on the intermediary metabolism of the rat for comparison.

These two problems are related but in terms of an approach to research in hibernation they should not be confused. Eventually, the knowledge of the activities of various organ systems and the whole animal will be integrated with the facts of intermediary metabolism of cells and the transport state of the circulatory system. At present, in hibernation research, the activities of the systems and the whole animal are far better known than the intermediary metabolism of the various tissues. It is important to bring the knowledge of the cellular metabolism of hibernating animals, both in and out of hibernation, to the level where such integration in the total economy of the animal is permissible.

We have used the golden hamster, *Mesocricetus auratus*, for this study of tissue metabolism. In recent years it has been recognized that there is not a single type of hibernation but a spectrum of hibernating types among the mammals. Of these the golden hamster has its own peculiar pattern which differs from that of certain other hibernating mammals such as the woodchuck and ground squirrel. Some of these differences are the

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prolonged sojourn in the cold (8-12 weeks) preceding hibernation for the hamster, and frequent arousals at which time food intake is necessary for survival if hibernation is entered again (Lyman and Leduc, 1953).

Our interest in the golden hamster is largely confined to the investigation of tissue metabolism and the possibilities of metabolic adaptations in cells. The long pre-hibernation period of cold exposure provides an opportunity to study the animal through many weeks of adaptation to cold stress. In a study of the adaptations of intermediary metabolism to cold stress and hibernation in the hamster we expect to find only an enhanced or diminished utilization of components and pathways, or alternative pathways already available to rodents in general. The oxygen consumption and response to substrates of tissues from cold-exposed and hibernating mammals is a good foundation for more detailed inquiry into intermediary metabolism by enzyme and substrate assay, radioisotope technique and cell particulate studies.

The Metabolism of Liver Slices from Non-Fasting Hamsters Exposed to Cold for Twelve Weeks and in Hibernation

Seven groups of male hamsters were studied in regard to body weight, liver oxygen consumption, and the response of liver to 2×10^{-2} M. succinate (Table 1). The control group was kept at room temperature while the cold-exposed and hibernating groups lived in a cold room at $5 \pm 1°C$. The animals were selected so that they would be approximately 6 months old when used. Each animal was weighed before use and killed by a sharp blow on the head. The liver and diaphragm were removed immediately and placed on moistened filter paper in a chamber over ice. Liver slices were cut with a Stadie-Riggs hand microtome to a thickness of 0.5 mm and kept cool in a moist chamber over ice until placed in the Warburg bath. The diaphragm was simply cut into small rectangles and treated in the same way as the liver slices. Each Warburg vessel contained 40-50 mg of tissue in a Krebs-Ringer phosphate buffer with the $CaCl_2$ reduced to three parts of a .055 M. solution. The oxygen consumption is expressed as QO_2 (microliters of O_2 per milligram of initial dry weight per hour at 37°C). Substrate was added from a sidearm after 30 minutes of endogenous respiration. For the determination of dry weight, slices comparable to those used for

TABLE I

Experimental Conditions	Body Weight (gm)	Liver				Diaphragm	
		% Dry Weight of Tissue	QO_2 Endogenous	QO_2 Succinate (2x10-2M.)	% Increase by Succinate	% Dry Weight of Tissue	QO_2 Endogenous
Control	153.2	29.8±.7*	4.21±.26	6.78±.07	61	22.7±1.8	5.93±.24
1 week at 5°C	119.5	31.2±.7	5.10±.43	8.72±.10	71	23.5±0.6	8.02±.29
3 weeks at 5°C	113.2	29.4±.9	5.45±.40	9.01±.46	65	23.9±0.2	8.37±.45
6 weeks at 5°C	106.6	30.9±.5	6.04±.14	9.39±.35	55	23.2±0.6	7.47±.19
9 weeks at 5°C	111.6	28.6±.9	6.81±.60	10.01±.59	58	22.8±0.6	9.46±.28
12 weeks at 5°C	105.6	30.7±.1	5.54±.10	8.94±.27	61	22.8±0.3	8.72±.15
Hibernation at 5°C	92.0	31.9±.9	5.33±.34	9.46±.45	77	24.0±0.8	9.41±.25

* S.E. 6 animals in each group.

Table 1. The body weight, percentage dry weight of tissues, endogenous oxygen consumption and response to succinate of liver and diaphragm from hamsters exposed to cold over a period of twelve weeks and in hibernation.

the determination of oxygen consumption were dried to a constant weight at 99°C. During the weeks of cold exposure there was a progressive loss in body weight. By the end of the first week, 21 per cent of the initial body weight was lost. Between the ninth week and hibernation, a weight loss of 17 per cent occurred. By the time the animals entered hibernation they had lost 40 per cent of their initial body weight.

There is no statistically significant difference in the dry weight of the liver tissue throughout the weeks of cold exposure and in hibernation (Table I). At the end of one week at 5°C the endogenous respiration of the liver had increased 21 per cent over that of the animals kept at room temperature. The oxygen consumption increased until the ninth week and then dropped to the level found in early cold exposure. Shortly after this the animals hibernated. The oxygen consumption of liver slices taken from hibernating animals that were allowed to "arouse" at 37°C is not significantly greater than that found in slices from animals exposed to cold for the first three weeks. This means that the pattern of metabolism in "arousing" liver slices is not one which produces an excess of oxidative processes beyond those already established in the cold-exposed animal. On exposure to cold, therefore, the oxidative metabolism of the liver increases 21 to 25 per cent over that of the control animals and no further increase is achieved during "arousal" of the tissue.

At the sixth and ninth weeks the endogenous respiration was highest, and at this time the addition of succinate had the least effect. The response of liver slices from control animals to the addition of succinate was similar. Slices from one-week cold-exposed animals and from hibernating animals gave a greater response to succinate, namely a 71 and 77 per cent increase. You and Sellers (1951) found an increase in liver succinoxidase in rats exposed to cold for several weeks while Campbell *et al.* (1958) found a similar increase in coenzyme A content of the liver of rats exposed to cold. The coenzyme A increase occurred within one day and persisted for 24 days of cold exposure. It is possible that there is an increase of these factors in the liver of hamsters exposed to cold. This, however, would not answer the entire problem of the response of liver to succinate since it is difficult to believe that these factors would increase initially, fall at twelve weeks of cold exposure and then increase again during hibernation. It is more likely that the enzyme and coenzyme content of the hamster liver remains high during cold exposure,

and the liver from one-week cold-exposed animals and hibernating animals is low in Krebs' cycle substrate. An increase in enzyme and coenzyme content of the tissues of cold-exposed hamsters should not be unexpected in light of the great increase in food consumption, as shown by Farrand and Folk (1957). The discussion by Potter (1958) on the nature of feedback mechanisms in enzyme adaptation describes the general effect of the increase of metabolites on the sequence of DNA to RNA to enzyme.

The Effects of Fasting on the Metabolism of Liver from Cold-Exposed Hamsters

In order to demonstrate more clearly alterations in the metabolism of liver from hamsters exposed to cold, an experiment based on a comparison of fasting and non-fasting was designed. A group of twelve male hamsters was kept at room temperature. Half of these were fasted for 24 hours before use but not denied water. Another group of twelve males was kept in the cold room ($5 \pm 1°C$) for three weeks. Half of these were fasted for 24 hours in the cold but not denied water. The effects of fasting, in these two groups, on the endogeneous metabolism of liver and its response to 2×10^{-2} M. succinate and 200 mg per cent glucose are presented in Table II. The data for the six hibernating hamsters were taken from the previous experiment. The tissue was treated as described in the previous section and substrates were added after 30 minutes of endogenous respiration.

There was no significant difference in the amount of dry material present or in the endogenous respiration of the liver from fasted and non-fasted control animals. In other words, 24 hours of fasting at room temperature does not affect the level of oxidative metabolism. The liver from both the fasting and non-fasting control animals responded to succinate with increases of 78 and 95 per cent oxygen consumption. Fasting, therefore, affected the tissue response to succinate to a slight extent. The addition of 200 mg per cent glucose to liver from fasted control animals had no effect on oxidative metabolism. These data suggest that liver from fasted control animals had some adaptation toward the greater use of a Krebs' cycle intermediate.

The endogenous respiration of liver from fasted, cold-exposed animals is significantly lower ($P<.01$) than that of the non-fasting cold-exposed animals. The oxygen consumption of the liver from these fasted animals had fallen to that of the control

TABLE II

Group	Experimental Conditions	% Dry Weight of Tissue	µl. O_2/Mg Dry Weight			% Increase by Substrate	
			30 mins	60 mins	90 mins	30 mins of Substrate	60 mins of Substrate
Non-Fasted Control	No substrate	29.9±0.1	2.32±.13*	4.21±.26	6.17±.43		
	2×10^{-2} M. Succinate		2.25±.12	6.78±.07	10.98±.29	61	78
24 hr Fasted Control	No substrate	31.0±0.9	2.43±.15	4.51±.32	6.52±.50		
	2×10^{-2} M. Succinate		2.47±.15	7.38±.21	12.09±.25	64	85
	200 mg. % Glucose		2.43±.09	4.51±.42	6.76±.42	0	0
3 weeks at 5°C, Non-Fasted	No substrate	29.4±1.0	2.98±.23	5.45±.40	8.07±.54		
	2×10^{-2} M. Succinate		2.97±.24	9.01±.46	14.89±.79	65	85
3 weeks at 5°C, 24 hr Fasted	No substrate	32.4±0.5	2.42±.14	4.42±.15	6.71±.18		
	2×10^{-2} M. Succinate		2.39±.18	8.54±.15	14.66±.35	93	118
	200 mg. % Glucose		2.52±.13	4.41±.21	6.60±.28	0	0
48-60 hrs Hibernation at 5°C	No substrate	31.9±0.9	2.79±.06	5.33±.34	8.04±.52		
	2×10^{-2} M. Succinate		2.73±.09	9.46±.45	15.76±.78	77	96

* S.E. 6 animals used for each experimental condition.

Table II. The percentage dry weight of tissue, endogenous oxygen consumption and percentage increase in oxygen consumption due to substrate for liver slices from fasted and non-fasted hamsters at 25°C, 5°C and in hibernation.

animals. The addition of glucose did not repair the drop in metabolism. The liver from these fasted, cold-exposed animals gave the greatest response to succinate (118 per cent) but none to glucose. These data show that both fasting at room temperature or fasting after three weeks in the cold room reduced the level of Krebs' cycle activity.

The endogenous respiration of liver from hibernating hamsters is similar to that from the non-fasting, cold-exposed animals. The response to succinate, however, is greater and this suggests a metabolic state intermediate between the non-fasted and fasted, cold-exposed animals. The animals used in this study had been in hibernation for only two or three days and were not very experienced performers. An experiment with one animal that had been in and out of hibernation regularly for a month gave different results. In this case the endogenous respiration of the liver was lower than that of the inexperienced animals and the response of the liver to succinate (128 per cent) was similar to that of a fasted, cold-exposed animal.

The Metabolism of Diaphragm from Non-Fasted Hamsters Exposed to Cold for Twelve Weeks and in Hibernation

The effect of sojourn in the cold ($5 \pm 1°C$) on the respiration of diaphragm from non-fasting animals for a period of twelve weeks and in hibernation are presented in Table I. These are the same animals on which the liver work was done and therefore these tissues can be compared directly. The diaphragm was cut into small rectangles and treated in the same way as the liver slices.

There was no statistically significant difference in the percentage of dry material of the diaphragm throughout the weeks of cold exposure. Respiration had increased 35 per cent (as compared to 21 per cent for liver) above that of the control animals after one week in the cold and was at this higher level at the third week. At the sixth week the respiration fell 12 per cent below that of the third week. Most of the animals in our colony become sleepy after six weeks in the cold and a few hibernate. The drop in respiration may be related to this phenomenon. This was followed by a marked increase in oxygen consumption of diaphragm at the ninth week. By the twelfth week respiration fell below that of the ninth week. After the twelfth week animals in the colony began to hibernate.

Diaphragm taken from hibernating animals and allowed to "arouse" at 37°C had an oxygen consumption 8 per cent above

the twelfth week prehibernation value, 17 per cent above tissue from animals exposed to cold for one week and 60 per cent above the value for control animals. Diaphragm, therefore, is capable of an impressive increase in oxygen consumption as the animal is taken from room temperature to an environment of 5°C. Considering its high level of oxygen consumption in the chronic cold-exposed animal it is remarkable that the diaphragm is capable of a further 8 per cent increase when "arousing."

The Effects of Fasting on the Metabolism of Diaphragm from Cold-Exposed Hamsters

The diaphragm was taken from the same animals used in the fasting experiment with liver, and the data are given in Table III. Fasting did not alter the endogenous metabolism of diaphragm from animals living at room temperature nor did the addition of glucose have any effect on oxygen consumption. Diaphragm taken from fasted or non-fasted cold-exposed animals had the same initial oxygen consumption. The oxygen consumption of the diaphragm from fasted animals began to fall off at 60 minutes, and by 90 minutes was significantly lower than that from the non-fasted animals. This fall in oxygen consumption was repaired by the addition of glucose but not increased beyond that of the non-fasted, cold-exposed animals.

In the intact mammal there is an intimate metabolic relationship between liver and muscle. A comparison of the respiration of liver and diaphragm from the fasted, cold-stressed animals suggests that the liver has supplied the diaphragm with glucose at the expense of its own oxidative metabolism. These results support the conclusions reached by Lyman and Leduc (1953) who used direct analyses for glycogen in liver, muscle and heart, and glucose in blood.

The Metabolism of Kidney Cortex from Cold-Exposed and Hibernating Hamsters

The endogenous oxygen consumption and the response of kidney cortex to citrate (7×10^{-3} M.), pyruvate (7×10^{-3} M.) and succinate (1×10^{-2} M.) was determined for four groups of hamsters (Tables IV, V). The control animals were kept at room temperature and the groups in the cold (5 ± 1°C) were studied at three days and twelve weeks of cold exposure, and in hibernation. Except for the hibernating animals, all were fasted for 24 hours before use. The animals were killed by a sharp blow on the head, the kidneys removed and decapsullated and demedullated in a

TABLE III

Group	Experimental Conditions	% Dry Weight of Tissue	µl. O_2/Mg Dry Weight		
			30 min	60 min	90 min
Non-Fasted Control	No substrate	22.7±1.8*	3.18±.13	5.93±.24	8.50±.34
24 hr Fasted Control	No substrate	24.6±0.7	3.14±.15	6.11±.29	8.67±.37
	200 mg. % glucose		3.35±.12	6.22±.32	8.76±.52
3 weeks at 5°C, Non-Fasted	No substrate	23.9±0.2	4.27±.26	8.37±.45	12.08±.67
3 weeks at 5°C, 24 hr Fasted	No substrate	22.9±0.6	4.25±.20	7.72±.26	10.88±.44
	200 mg. % glucose		4.44±.21	7.89±.39	11.66±.55

* S.E. 6 animals used for each experimental condition.

Table III. Percentage dry weight, endogenous respiration and response to substrate for diaphragm from fasted and non-fasted hamsters at 25°C and 5°C.

moist chamber over ice. Cortical slices were cut to 0.4 mm thickness by hand and treated in the same way as the liver and diaphragm. The medium was a modified Krebs-Ringer solution at pH 7.3 with Tris buffer in a final concentration of .03 M. Comparable tissue slices were used to determine the dry weight and the nitrogen content. The tissue was dried to a constant weight at 99°C. Substrates were added from sidearms after 30 minutes of endogenous respiration.

Blood for ion analysis was withdrawn by cardiac puncture and allowed to clot, and bladder urine was withdrawn by hypodermic at the same time. The concentrations of sodium and potassium in blood and urine were determined with a Coleman flame photometer and expressed as milli-equivalents per liter (Table VI).

The endogenous oxygen consumption of kidney cortex from control animals is approximately 2½ times greater than that of diaphragm, and 3 times that of liver tissue (Fig. 1). During cold exposure, the difference between kidney cortex and diaphragm is slightly reduced but this difference is maintained between the kidney cortex and liver tissue. After three days in the cold room, the kidney cortex had an endogenous oxygen consumption significantly higher ($P<.01$) than that of the control, the twelve week cold-exposed, or hibernating animals. By three days of cold exposure the dry weight of the cortex was greater, the total wet weight of the kidney had increased 25 per cent and the animals had lost 16 per cent of their initial body weight. Associated with these changes was a 17 per cent increase in 12-hour urine volume and an increase in the output of potassium. There was no change in serum sodium or potassium nor in any ratio between these two ions except the urinary sodium to potassium ratio which fell from 0.9 to 0.6. The greatly increased water load had forced the kidneys to work harder in order to maintain the blood ion balance. The excess loss of potassium would be the result of passive removal with the water (Smith, 1956). Hastings *et al.* (1952) working with rat liver found that a potassium-rich medium facilitated the conversion of glucose-6-PO_4 to glycogen while a sodium-rich medium was inhibitory to this reaction. If potassium lost from the blood were being replaced by a movement of potassium out of tissues with an inward movement of sodium this would provide a stimulus for glycogenolysis. This point should be specifically investigated since Masoro *et al.* (1954) found that one day of cold exposure drastically reduced the glycogen level of non-fasted rats.

TABLE IV

Group	No. of Animals	Weight of Animal (gms)	Weight of Kidney (mg)	% Dry Weight of Tissue (mg)	ul. O_2 Consumption/Mg Dry Weight		
					30 mins	60 mins	90 mins
Control	12	129.5±2.8*	459±20	23.4±0.2	7.12±.05	13.68±.32	20.24±.69
3 days at 5°C	11	108.5±3.3	574±28	25.7±0.3	8.15±.21	15.41±.35	22.09±.56
12 weeks at 5°C	11	95.5±4.3	637±23	23.8±0.5	7.51±.23	14.57±.30	21.12±.71
Hibernation	12	83.5±3.8	644±35	22.1±0.6	6.47±.25	12.50±.33	17.99±.49

* S.E.

Table IV. The body weight, kidney wet weight, percentage dry weight of tissue and endogenous oxygen consumption for kidney cortical slices from hamsters at 25°C, 5°C and in hibernation.

TABLE V

Group	No. of Animals	QO_2	% Increase of O_2 Consumption**		
			Citrate $7x10^{-3}$M.	Pyruvate $7x10^{-3}$M.	Succinate $1x10^{-2}$M.
Control	12	13.68±.32*	49	21	84
3 days at 5°C	11	15.41±.35	47	23	87
12 weeks at 5°C	11	14.57±.30	40	21	91
Hibernation	12	12.50±.33	55	34	128

* S.E.

** At end of 60 minutes respiration with substrate.

Table V. The QO_2 and percentage increase in oxygen consumption due to citrate, pyruvate and succinate for kidney cortical slices from hamsters at 25°C, 5°C and in hibernation.

By twelve weeks of cold exposure the oxygen consumption of kidney cortex was only slightly higher than the control level. The animals had lost 26 per cent of their initial body weight and the wet weight of the kidney had increased 39 per cent over that of the control animals. The dry weight of the tissue had returned to control value. After three weeks in the cold the sodium and potassium ratios were similar to those of the control animals but the urine volume remained high. The twelve-week animals may have had a similar ion pattern but we have no data for them.

The QO_2 of kidney cortex taken from hibernating animals and measured in a Warburg at 8°C, which was the internal body temperature of our hibernating animals, was 1.38 ±.05 (Table VII). This is 9 per cent of the oxygen consumption obtained from kidney cortex taken from fasted, twelve-week, cold-exposed animals and measured at a temperature of 36°C. During hibernation the urinary sodium to serum sodium ratio was double that of the non-fasted, cold-exposed animals. The sodium content of the serum had fallen to the fasted control level and was high in the bladder urine. Sodium had been lost from the blood and had not been replaced. The kidney tubules were probably capable of little or no sodium return. The serum potassium remained high in spite of the low activity of the kidney and the high concentration of potassium in the bladder urine. Obviously, unlike the sodium, the potassium of the serum was being replenished. Most mammalian cells have a higher potassium than sodium concentration and this is accomplished both by the Gibbs-Donnan effect and by active transport of one or both ions. Cooling the tissue would greatly reduce the active transport mechanism.

It is interesting to speculate on what this might mean in regard to the glycolytic cycle and to the prominence of the T wave in the electrocardiograms of arousing hamsters (Chatfield and Lyman, 1950). As the hamster arouses the body temperature rises and the ions would gradually return to their normal relationships. During arousal, however, a lower potassium in liver and muscle would encourage glycogenolysis and the high serum potassium would explain the prominent T wave of the heart.

The endogenous oxygen consumption of kidney cortex from hibernating hamsters measured at 36°C was lower than that from any of the other groups and fell off significantly by 90 minutes. In the intact animal the kidney cortex is therefore

TABLE VI

Experimental Group	No. of Animals	Serum (meq./L) Na	Serum (meq./L) K	Urine (meq./L) Na	Urine (meq./L) K	U_{Na}	S_{Na}	S_K	U_K	S_K	S_{Na}	U_{Na}	U_K
Normal Fasted	7	150±2.1*	5.1±0.3	52±9.7	53±5.5	0.3	0.09	0.03	0.9				
3 days at 5°C 24 hr Fasted	7	148±1.5	5.3±0.1	43±4.9	65±3.0	0.3	0.08	0.03	0.6				
3 weeks at 5°C 24 hr Fasted	6	144±2.4	5.2±0.2	39±8.9	43±4.4	0.3	0.12	0.03	0.9				
3 weeks at 5°C Non-Fasted	8	163±3.6	7.2±0.7	118±7.0	228±19	0.6	0.03	0.04	0.6				
Hibernation	8	152±2.3	6.9±0.2	185±27	256±15	1.2	0.03	0.04	0.7				

* S.E.

Table VI. The concentrations and ratios of sodium and potassium in serum and bladder urine of fasted and non-fasted hamsters at 25°C, 5°C and in hibernation.

TABLE VII

Group	No. of Animals	Warburg Temperature	QO_2	Q_{10}
Control	12	36°C	13.68±.32*	2.1
	6	8°C	1.67±.04	
Hibernation	12	36°C	12.50±.33	2.1
	6	8°C	1.38±.05	

* S.E.

Table VII. The QO_2 and Q_{10} of kidney cortical slices from hamsters at 25°C and in hibernation.

dependent on exogenous substrate, such as glucose from the liver, in order to regain the high level of oxidative metabolism found in the chronic cold-exposed animals.

Since the kidney cortex has a very high oxygen consumption and accomplishes a great deal of work for the maintenance of homeostasis in the hamster and yet survives the low body temperatures of the hibernating animal, its Q_{10} is of interest (Table VII). The Q_{10} of tissue from fasted control animals is the same as that of the hibernating animals. Weiss (1954) suggested that a low Q_{10} such as he found for heart slices from rats might render an organ insensitive to changes in external temperature and thus provide it with viability. The hamster kidney cortex has a Q_{10} at the level of rat brain and according to our *in vitro* work at 8°C probably slowly oxidizes substrate during hibernation.

In considering the response of kidney cortex to substrates (Table V), the effect of the various substrates should not be compared with one another since their rate of entry into the tissue is different. There is a fall in the utilization of citrate at the twelfth week of cold exposure and a slight increase over control level in the arousing tissue. The response to pyruvate remains constant except in the tissue of hibernating animals where there was a 13 per cent increase over the control or cold-stressed animals. The response to succinate is slightly higher in cold-exposed animals and very profound in tissue taken from hibernating animals. The response of kidney cortex from hibernating animals to these substrates shows that oxidative mechanisms were unimpaired but that substrate was not as available for Krebs' cycle activity as in the active animals.

Changes in Body Weight Related to Oxygen Consumption of Liver, Diaphragm and Kidney

During prolonged exposure to cold the body weight of the hamsters decreased drastically, and they usually entered hibernation at weights between 80 and 95 grams. When the oxygen consumption of liver slices and diaphragm from non-fasted control, three-week and twelve-week non-fasted, cold-exposed animals, and animals in hibernation were plotted against their respective body weights a linear relationship was revealed (Fig. 1). The data on liver metabolism from the nine-week cold-exposed and hibernating hamsters and the data for diaphragm taken from six-week cold-exposed animals are exceptions. The

data for kidney cortex are taken from a different group of fasted animals and are plotted for control, three days, and twelve weeks of cold exposure, and for hibernation.

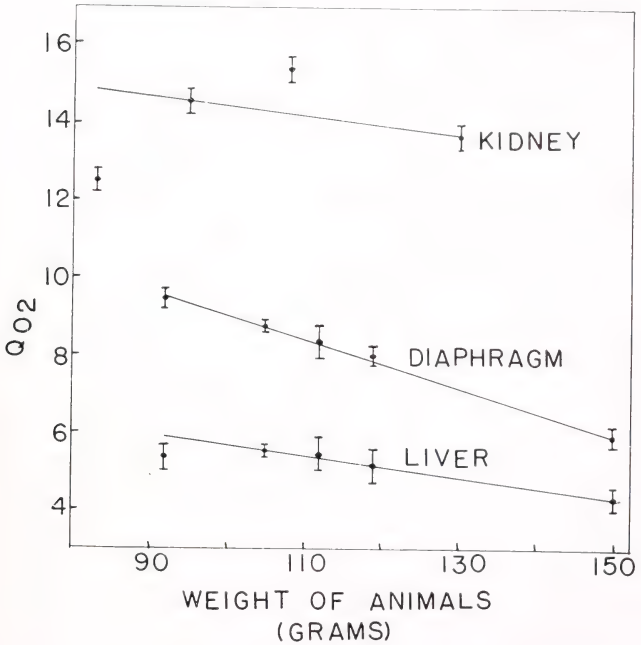

Fig. 1. The effect of weight loss in the cold on the oxygen consumption of liver and kidney cortical slices and diaphragm from hamsters of comparable age ($\frac{1}{1}$ = mean and standard deviation).

Weiss (1954) published similar data on oxygen consumption of visceral tissues from white rats exposed to cold (5 $\pm$1°C). His results were plotted against increasing weight as a measure of age, whereas the hamsters were chosen so that they would be approximately 6 months old when used. He obtained the same relationships as shown for the hamsters. Weiss attributed his results to the effect of age on oxygen consumption. With the

hamsters, the same effects were obtained as a function of weight loss only, in a similar cold environment. When working with six-week old rats in the cold for 10 days his results with diaphragm are similar to those for hamster diaphragm. On the other hand, cold exposure did not increase the oxygen consumption of diaphragm from six-month old rats. Rat liver QO_2 increased significantly by the tenth day of cold exposure in both the six-week old and six-month old rats. Hamster liver did not achieve a QO_2 comparable to that of the six-week old rats until 3-6 weeks of cold exposure. Hannon (1958) found that the peak QO_2 of rat liver occurred at 4 weeks of cold exposure and then fell off during subsequent weeks whereas the QO_2 of hamster liver increased gradually until 9 weeks of cold exposure.

Discussion

There is evidence from this work that the general oxidative response of tissues of rats and hamsters exposed to cold is similar, but that the timing is different. This difference in timing may be correlated with an inability of the hamster to maintain its protein content.

The general interrelationships of liver, diaphragm and kidney of cold-exposed and hibernating hamsters are defined by this work. Although liver slices respond to cold exposure of the animal the liver does not indulge in excess oxidative metabolism when "aroused." The diaphragm from the same animals, however, produces not only a high QO_2 but provides excess oxidation during *in vitro* arousal. The diaphragm, therefore, utilizes its substrate stores for excess oxidative metabolism, and in the intact animal one could reasonably expect such extravagance to be constantly replenished by glucose from the liver.

The kidney is not a glycogen-storing organ and depends constantly on transported substrate. The fact that the QO_2 of arousing kidney cortical slices is significantly lower than that of the fasted control animals demonstrates that the kidney cannot return to an effective oxidative level unless supplied by endogenous substrate. The kidney maintains many aspects of mammalian homeostasis under extreme stresses. Its metabolism and function in hibernating mammals should be studied more intensively.

The high potassium and lowered sodium content of the serum of hibernating hamsters is interpreted here as the result of the depression of the active transport systems of the kidney, the

erythrocytes and all the tissues of the body. It may result largely from an exchange between erythrocytes and plasma and the actual content of tissues has yet to be determined. If these ions are shifting their equilibria between tissue and plasma as hibernation is entered and during arousal, temporary but highly significant effects may be produced in the biochemical and biophysical state of the animals.

The 40 per cent loss of initial body weight in the prehibernating hamster is a striking phenomenon. According to Zimny and Tyrone (1957) *Citellus tridecemlineatus* loses 20 per cent of its body weight before entering hibernation. Masoro *et al.* (1957) described a 20 per cent loss of body weight for white rats living at 0-2°C for four months. It seems unlikely that oxidation of fat could account entirely for the large weight loss of the hamster. Previous to hibernation the hamster is frail in appearance and seems to have drawn heavily on its muscle protein.

It is well known that fats and carbohydrates have a protein sparing action (Munro, 1951). We have found that chronic cold-exposed and hibernating hamsters have good fat deposits and that the liver of the hibernating hamster has 50 per cent of the lipid content of the control animals. It is possible that in the hamster carbohydrate and fatty acid oxidation cannot or does not exert sufficient sparing action.

It is our intent in the future to investigate by radioisotope technique the interrelationships among fat, carbohydrate and protein metabolism in the tissues of cold-exposed and hibernating hamsters. The large loss in body weight suggests an important contribution through gluconeogenesis to the total economy of the cold-exposed hamster during its preparation for hibernation. Furthermore, the hormonal control of the integrated fat, carbohydrate and protein metabolism of the cold-exposed and hibernating mammal will have to be elucidated before a complete understanding of the metabolic state of hibernating mammals is achieved. A preliminary step in this direction would be a quantitative estimation of the actual circulating level of various hormones in the cold-exposed and hibernating mammal particularly from a comparative point of view.

Summary

The QO_2 of liver slices and diaphragm was increased significantly when taken from non-fasted hamsters exposed to cold for one, three, six, nine and twelve weeks.

The greatest QO_2 for liver slices and diaphragm from non-fasted hamsters occurred at nine weeks of cold exposure.

At six weeks, the QO_2 of diaphragm of non-fasted hamsters fell significantly. This may be correlated with early attempts at hibernation or with changes in heat loss due to some factor such as pelage increase.

The QO_2 of liver slices and diaphragm of non-fasted hamsters at twelve weeks of cold exposure fell significantly below their QO_2 at nine weeks. Shortly after this the animals hibernated.

The QO_2 of kidney cortical slices from fasted hamsters was highest when measured after 3 days of cold exposure and fell to slightly above control level at twelve weeks.

The QO_2 of diaphragm was increased significantly when "arousing" *in vitro* as compared to its prehibernation value whereas the QO_2 of kidney and liver slices fell below their prehibernation value during *in vitro* "arousal."

Succinate (2×10^{-2} M.) produced the same increase (85 per cent) in oxygen consumption of liver slices from non-fasted 3-week cold-exposed hamsters as with liver slices from fasted control animals. With liver slices from fasted 3-week cold-exposed animals the oxygen consumption was greatly increased (118 per cent) by succinate. The response of liver from hibernating animals (96 per cent) is below this value for inexperienced hibernating animals or higher (128 per cent) for an experienced individual.

Liver slices from fasted control and fasted 3-week cold-exposed animals did not increase their QO_2 in the presence of 200 mg per cent glucose. The oxygen consumption of diaphragm from fasted 3-week cold-exposed animals fell off at 90 minutes of incubation and was repaired by the addition of 200 mg per cent glucose. Glucose had no effect on the oxygen consumption of diaphragm from fasted control animals.

There is little difference in the response of kidney cortical slices from control, 3-day and 12-week cold-exposed fasted hamsters to citrate (7×10^{-3} M.), pyruvate (7×10^{-3} M.) and succinate (1×10^{-2} M.) All these substrates significantly increased the QO_2 of slices from hibernating animals. The two Krebs' cycle intermediates had the most effect on the QO_2 of "arousing" kidney cortical slices.

The Q_{10} of kidney cortical slices from fasted control and experienced hibernating animals is the same and is at the level of Q_{10} for brain of the six-month old rat.

At 8°C, *in vitro* kidney cortical slices from hibernating animals have a QO_2 only slightly below that of slices from fasted control animals at the same temperature. This relationship persisted when these tissues were raised to 36°C *in vitro* but was repaired either by a substrate of the glycolytic cycle or of the Krebs' cycle.

At the body temperature of hibernation, the oxygen consumption of the kidney cortex *in vitro* was no higher than 9 per cent of that from the active cold-exposed hamster and is likely lower than this in the intact animal where oxygen may not be as available as in a Warburg vessel. Its function in active transport of ions would be insignificant. Sodium had accumulated in the bladder and fallen in the serum to the level of the fasted control animals. Potassium had both a high concentration in the bladder and a serum concentration at the level of the active non-fasted cold-exposed animals. The excess potassium had probably moved out of the tissues.

There was a linear relationship between the weight loss from prolonged cold exposure and the QO_2 of liver and diaphragm from non-fasted hamsters of the same age. This same relationship was true for rats in the cold whose weight and age were increasing simultaneously.

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DISCUSSION FOLLOWING DENYES' PAPER

STRUMWASSER asked if DENYES had any data on the effect of age *per se* on the ability of the hamster to hibernate and perhaps on the ability of the whole animal to show cellular adaptation to cold. DENYES replied that she did not, although she had a 2-year hamster that did not hibernate after the first year. She also observed that some animals apparently attempt to hibernate without success and never try again.

SOUTH noted for a fact that, after a prolonged sojourn in the cold room and after periodic hibernating spells, hamsters stop hibernating in the cold.

DENYES remarked that she believes the hamster is an animal in dangerous metabolic balance when in a cold environment, probably with considerable protein, fat, and carbohydrate exchange involved. She believes there is a great shifting of hormonal relationships with a state finally reached conducive to hibernation. This state may never be reached again.

XXIV

PHOSPHATES AS RELATED TO INTERMEDIARY METABOLISM IN HIBERNATORS

By Marilyn L. Zimny
Louisiana State University
School of Medicine
New Orleans, Louisiana

In 1949 Wollenberger gave a review of work on the energy metabolism of the failing heart and reported his original research. By means of Starling heart-lung preparations in dogs the intermediary metabolism of the energy-rich phosphate content was studied under various experimentally induced conditions of cardiac failure. Later he reported that the rate of utilization of phosphate bond energy by the heart is a function of frequency of beat (Wollenberger, 1951).

Work underway in neighboring laboratories investigating a possible mechanism of cardiac glycoside action (Proctor *et al.*, 1955) and the high-energy phosphate content of different areas of the dog heart (Mulder *et al.*, 1956) stimulated my interest in phosphates and cardiac metabolism.

The heart rate, respiration rate, and body temperature of active and hibernating ground squirrels were compared by Johnson (1931), and those of active and hibernating marmots by Benedict and Lee (1938). The decrease in heart rate which was found during hibernation made it seem that the high-energy phosphate compounds, adenosine triphosphate and phosphocreatine, should be investigated and correlated with the energy cycle of glycolysis as a means of interpreting the metabolic adaptations of hibernators.

Early reports of analytical work on hibernating mammals pertained to blood glucose and liver glycogen (Dubois, 1896; Weinland and Riehl, 1908; Weinland, 1925; Endres and von Frey, 1930; Suomalainen, 1935) but relatively little material was available on phosphate compounds (Ferdmann and Feinschmidt, 1932; Feinschmidt, 1936). Recently, the glycogen content of various organs of hibernants has been adequately reviewed by Lyman and Chatfield (1955), Eisentraut (1956) and Kayser (1957). There is general agreement that hypoglycemia occurs during hibernation, but the degree reached varies from species

to species, and that reconstruction of cardiac muscle glycogen during hibernation takes place at the expense of skeletal muscle and liver glycogen. Our studies of phosphate and carbohydrate compounds in the thirteen-striped ground squirrel, *Citellus tridecemlineatus*, include the heart, skeletal muscle and liver.

This investigation during the past eight years has included animals that were sacrificed (1) following a period of 3 to 5 days of uninterrupted hibernation; (2) following a period of 30 days of uninterrupted hibernation; and (3) following stimulated arousal periods of 7.5, 15 and 30 minutes. The control animals were maintained at an environmental temperature of 25-27°C while the experimental animals were kept in an environmental temperature of 3-5°C. Records of body weight, heart rate (EKG tracings), respiration rate, and rectal temperatures (thermocouple measurements) were kept on several animals for aids in interpretation.

Glycogen determinations in early phases of the project were made according to Pflüger's method as modified by Good *et al.* (1933), and Somogyi's method as modified by Nelson (1944) for glucose hydrolyzed from glycogen. As work progressed it was desirable to analyze the same tissue sample for other compounds in addition to glycogen. Since the ground squirrel heart is small in size, another glycogen method was needed in order to carry out this procedure. Therefore, glycogen was determined by the method of Kemp and van Heijningen (1954). Tissue lactate and pyruvate have been determined by the methods of Miller and Muntz (1938) and Lu (1939), respectively. Determinations of phosphate fractions were based upon the Fiske-Subbarow reaction as used by Wollenberger (1947). The symbols for the various phosphate fractions are as follows: adenosine polyphosphate (APP), adenosine triphosphate (ATP), adenosine diphosphate (ADP), phosphocreatine (PC), and inorganic phosphate (IP).

Cardiac Muscle

Phosphocreatine was described in skeletal muscle by Fiske and Subbarow in 1929, and in the same year Pohle (1929) reported that muscle adenosine phosphoric acid and also a hexosemonophosphoric acid had been isolated from the ox heart. Ferdmann and Feinschmidt (1932) made an extensive study of the chemical changes in muscle in a small number of hibernating ground squirrels, *Citellus guttatus*, and marmots, *Arctomys bobac*. They reported that during hibernation the acid-soluble

phosphate of the heart decreased with decreasing ortho-phosphate and phosphocreatine. Correlation was suggested between the activity of the heart and ortho-phosphate content due to the finding that this compound decreased with decreasing heart rate.

Early studies relating to phosphoric acid metabolism and the total metabolism of the heart are reviewed by Cruickshank (1936). By this time it was apparent that the amount of phosphorus produced by the heart was dependent on the rate of contraction. Although the studies of Lundsgaard (1930) had shown that muscular contraction derived its energy from phosphagen hydrolysis, and Lohmann and Schuster (1935) had studied the presence of adenylpyrophosphate and adenosine diphosphate in heart muscle, it was still questionable whether or not cardiac muscle metabolism paralleled that in skeletal muscle. The interest in the relationship of phosphates in cardiac metabolism was derived early from studies of the physiology of the failing heart and later from the pharmacological action of various drugs upon the heart. Szent-Györgyi (1947) reported that the actomyosin obtained from cardiac muscle was indistinguishable from that extracted from skeletal muscle but still analyses of cardiac muscle action based on cellular and molecular changes were wanting when Wollenberger reported that heart failure can occur in the presence of normal amounts of ATP and PC in the myocardium (1947); that the heart has low ability for anaerobic recovery due to a small PC reserve and low glycolytic power (1949); that the heart resynthesizes ATP at the expense of PC (1949); and that the rate of utilization of energy-rich phosphate by the heart is a function of heart rate (1951).

When the phosphate compounds, i.e., inorganic phosphate (IP), adenosine polyphosphate (APP), considered in our work as adenosine triphosphate (ATP), and phosphocreatine (PC), were first studied in the ground squirrel heart, it was found that ATP decreased and PC increased approximately to the same degree and IP remained unchanged (Zimny, 1956). As work continued it was soon apparent that this was not correct. In these early experiments procedures involving handling of the hibernating animal, such as weighing and taking electrocardiographic tracings prior to sacrifice, introduced a time factor which proved to influence the final results. During later experiments the hibernating animals were sacrificed and the tissue removed within the first 30 seconds of handling the animal. The values obtained from these animals, showing a maintenance of high-energy phosphate and a significant decrease of 20 per cent

in IP, we believe, are representative of true hibernation (Zimny and Gregory, 1958b). It is inferred from the work of Bing (1955) and Wollenberger (1947) that utilization and generation of phosphate bond energy have reached an equilibrium at a greatly reduced heart rate and work load. As hibernation is

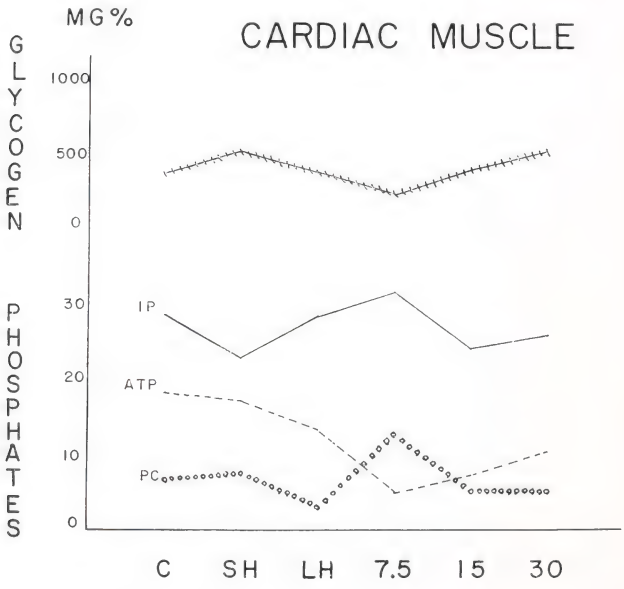

Fig. 1. Concentrations of glycogen and phosphates in cardiac muscle following 3-5 days of hibernation (SH); 30 days of hibernation (LH); and arousal periods of 7.5, 15, and 30 minutes as compared with controls (C).

prolonged from an uninterrupted period of 3-5 days to an uninterrupted period of 30 days, ATP and PC decrease significantly, with IP increasing toward the control value (Fig. 1) (Zimny and Gregory, 1959). Phosphocreatine shows a greater decrease than ATP because it is maintaining ATP to supply energy for the slowly beating heart.

Changes in these compounds take place quickly upon stimulated arousal following 3 to 5 days of uninterrupted hibernation,

especially in the heart(Zimny and Gregory, 1958b). Within 7.5 minutes glycogen decreases 55 per cent, IP increases 38 per cent, ATP decreases 70 per cent and PC increases 68 per cent. Within 15 minutes of arousal glycogen and ATP are increasing while IP and PC are decreasing, and by 30 minutes the phosphate levels are beginning to resemble the control pattern (Fig. 1). Cardiac muscle glycogen continues to increase. Therefore, the immediate use of ATP for energy is for stimulating glycolysis and is followed by PC transferring phosphate to ATP and possibly to other metabolic systems for the purpose of glyceneogenesis.

Cardiac muscle glycogen levels during the hibernating periods were studied and following five days of uninterrupted hibernation this compound had increased 60 per cent, indicating that the heart is storing carbohydrate for the thermogenic processes of arousal. Although glycogen in the heart decreased 36 per cent upon prolongation of the hibernating period to a month, the decrease did not influence the physiological state of the animal.

Lactate and pyruvate levels decrease during hibernation but the lactate-pyruvate ratio remains within the average control range for cardiac muscle of 9/1 (Zimny and Tyrone, 1957). The decrease in these compounds apparently is due to a slower rate of glycolysis with a decreased metabolic rate.

Skeletal Muscle

The pioneer investigations of intermediary metabolism in relation to carbohydrate and phosphoric acid were done on skeletal muscle (Fiske and Subbarow, 1927; Eggleton, 1929; Meyerhof, 1930; Milroy, 1931). In their study of the chemical changes in muscle of the hibernating animal, Ferdmann and Feinschmidt (1932) reported a decrease in phosphocreatine, inorganic phosphate, hexosemonophosphate, acid-soluble phosphate and total phosphorus in both the hibernating ground squirrel, *Citellus guttatus*, and the marmot, *Arctomys bobac*. Later studies by Feinschmidt (1936) revealed a significant reduction in ATP content and an increase in inorganic phosphate and free adenylic acid during hibernation.

The importance of PC and APP to the contractile system of muscle was emphasized by Kalckar (1941) in a review of the energetic oxidation-reduction reactions which occur during biological syntheses. Mention was also made that cellular structures may be acting as phosphate-transfer systems in the living

cell. Mommaerts (1950) labelled the splitting of PC as the energetic master reaction in normal muscle, and in 1951 Kaplan extensively described the thermodynamics and energy mechanisms of the phosphate bond. The work of Szent-Györgyi (1953) gives evidence that glycolysis furnishes energy for converting

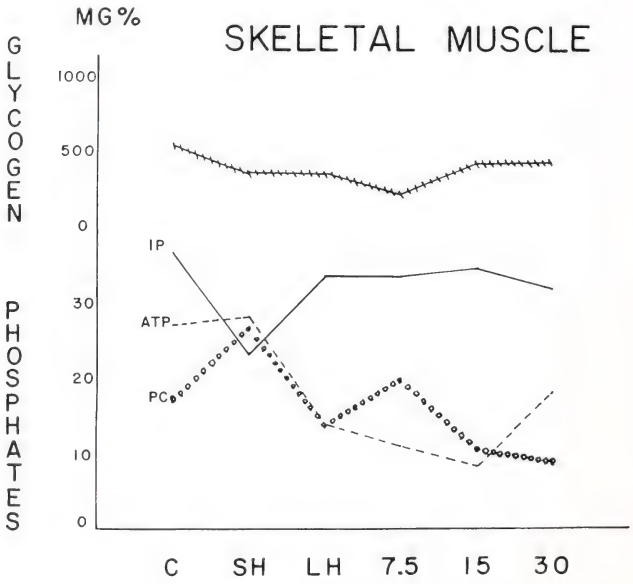

Fig. 2. Concentrations of glycogen and phosphates in skeletal muscle following 3-5 days of hibernation (SH); 30 days of hibernation (LH); and arousal periods of 7.5, 15, and 30 minutes as compared with controls (C).

ADP to ATP which breaks down during contraction, and PC may be rephosphorylating ADP held by contracted myosin. In 1954 Mommaerts stated that the purpose of metabolism is to generate ATP as it is used, and the findings of Uchida *et al.* (1954) supported the theory that ATP is the immediate source of energy for contraction, with PC splitting in the initial phase of muscle contraction. Correlation of the mechanical with the

chemical events which occur during muscle contraction, incorporating both the glycolytic and oxidative phosphorylating mitochondrial systems has recently been reviewed by Perry (1956) and by Buchthal *et al.* (1956).

Phosphate compounds in skeletal muscle samples from the early hibernation studies (Zimny, 1956) followed a pattern similar to those in cardiac muscle. Adenosine triphosphate decreased and PC increased to approximately the same degree with a small decrease in IP. Later investigation (Zimny and Gregory, 1958b) showed that ATP is maintained during hibernation, and PC increases apparently at the expense of decreasing IP and glycogen. When the hibernating period is prolonged to 30 days, both ATP and PC decrease but the ATP-PC ratio remains unchanged at 1/1 (Fig. 2). Immobility for the extended period explains this combined decrease (Zimny and Gregory, 1959).

Adenosine triphosphate is used as the immediate source of energy during arousal with PC affecting resynthesis (Zimny and Gregory, 1958b). Within 7.5 minutes of arousal PC decreases 46 per cent indicating a phosphate transfer forming ATP which then shows a 114 per cent increase by the end of 30 minutes of arousal (Fig. 2). As might be expected, skeletal muscle activity lags behind that of cardiac muscle during arousal. After 15 minutes of arousal the heart rate had increased 192 per cent, a gain of 71 beats, and after 30 minutes, it increased 644 per cent, a gain of 174 beats. At neither time was the increase in skeletal muscle activity in any way comparable. Glycogen increases after 15 minutes of arousal and continues to do so after 30 minutes. Once again a possible explanation may be that of glyconeogenesis.

Skeletal muscle glycogen decreases during 3 to 5 days of hibernation and continues decreasing with increasing length of the hibernation period. This decrease may be due to increasing the stores of cardiac glycogen or a metabolic response to low temperature prior to hibernation.

Lactate and pyruvate decrease in skeletal muscle during hibernation but the ratio between the two compounds remains within the average control range of 13/1 (Zimny and Tyrone, 1957). The decrease is apparently due to a slower rate of glycolysis at a decreased metabolic rate.

Liver

Liver phosphate studies are few because the early work on phosphates was centered upon the chemical changes associated

with muscle contraction. A liver homogenate was used as a source of adenylpyrophosphatase in studies pertaining to muscle (Jacobsen, 1931), and studies of changes in liver phosphates as a result of experimental conditions were related to fasting and

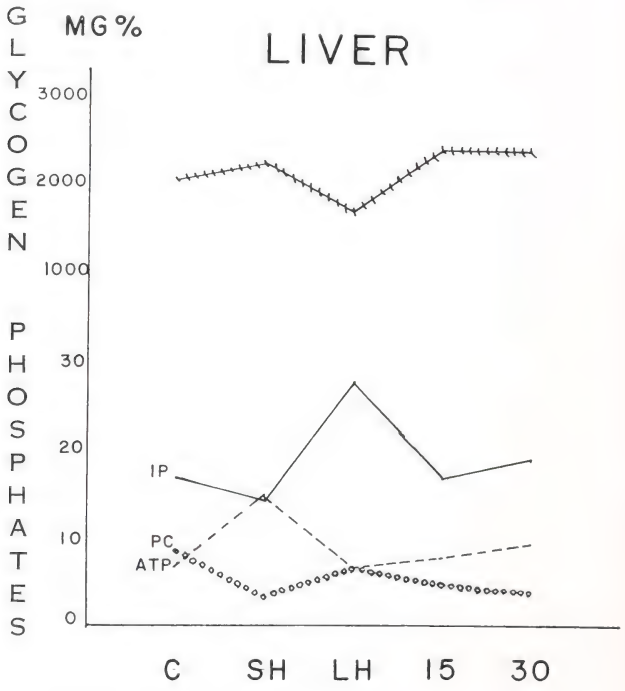

Fig. 3. Concentrations of glycogen and phosphates in liver following 3-5 days of hibernation (SH); 30 days of hibernation (LH); and arousal periods of 15 and 30 minutes as compared with controls (C).

the effects of various diets (Flock *et al.*, 1936). In 1939 Hevesy found a much larger turnover of labelled phosphorus in the liver, kidneys, and intestinal tract than in the muscles or brain of rats. Both Nelson *et al.* (1942) and Rapoport *et al.* (1943) found that the concentration of IP increased in the livers of fasted

rats, with a decrease in the easily hydrolyzable fractions. No explanation for the shift was given. The liver, as stated earlier, was among the first organs analyzed for glycogen in the hibernant and early work on carbohydrate metabolism is aptly reviewed by Cori (1931). More recent work is discussed by Soskin and Levine (1952).

In the liver of ground squirrels, adenosine triphosphate increases 121 per cent and PC decreases 62 per cent following 3 to 5 days of hibernation (Fig. 3). As the hibernating period is prolonged, ATP decreases 55 per cent and PC increases 107 per cent so that the values at this time are nearly equal. After a 15 minute arousal period following 3 to 5 days of uninterrupted hibernation, ATP has decreased 47 per cent and PC increases 44 per cent. By 30 minutes of arousal it is apparent that PC is transferring phosphate for ATP formation. During hibernation, the lessened metabolic demand allows ATP to accumulate for the purpose of supplying energy to the many liver functions which occur upon awakening (Zimny and Gregory, 1958b, 1959).

Liver glycogen increases during early stages of hibernation, shows a decrease after 30 days of hibernation and increases during arousal (Zimny and Gregory, 1958b, 1959). For want of any other explanation, the increase in tissue glycogen is attributed to processes of glyconeogenesis. Liver glycogen values during hibernation and arousal depend upon several factors. Variations in respiration rate during the hibernating period could influence the glycolytic rate. The length of time the animal had spent in the cold environment responding to calorigenic stimuli before entering hibernation should also be considered in addition to the composition of the diet and weight loss factors. Lastly, the method of sacrificing an animal can influence the tissue glycogen level as well as the levels of other compounds.

Both lactate and pyruvate levels decrease during hibernation, but as in the heart and skeletal muscle the lactate-pyruvate ratio remained within the control range of 10-20/1 (Zimny and Tyrone, 1957).

Related Studies

Brown fat. The process of glyconeogenesis has been mentioned as a possible explanation for increased glycogen levels in the tissues studied. With this in mind the interscapular brown fat pads of hibernated, aroused and control ground squirrels

were analyzed (Zimny and Gregory, 1958a). Hook and Barron (1941) found that although in most tissues the metabolism is reduced to a minimum during hibernation, brown adipose tissue still maintains one-third of its optimum activity in terms of respiratory action of tissue slices. Klar (1941) suggested that activity increases in this tissue in the hedgehog during the winter because the oxidation-reduction potentials of brown fat are greatly reduced during hibernation. In 1953 Karolewicz reported that brown fat of the hedgehog does not contain nutritious stores for the hibernation period, but that stored compounds are used in the pre-rutting period immediately after the animal has awakened. Further study (Karolewicz, 1956) showed that the glycogen content of brown fat is low in June and high in November with a daily rhythm during the summer months showing peaks of activity in the daytime and at 3:00 A.M. Recent histochemical and microchemical studies on the lipids of the bat, *Myotis lucifugus lucifugus* (Rémillard, 1958) revealed decreases in total weight, water content, total lipids, total cholesterol and total fatty acids during hibernation.

Our studies on the biochemical composition of brown fat in the ground squirrel showed an increase in glycogen and pyruvate and a decrease in lactate during hibernation and showed all three compounds decreasing during arousal (Zimny and Gregory, 1958a). Tissue lactate was high in the control group, lactate-pyruvate ratio of 56/1, when compared to the ratios in the other groups averaging 20/1. In fact the lactate-pyruvate ratio of brown fat in the control is much higher than ratios ever obtained for cardiac muscle, skeletal muscle, and liver which are in the range of 10-20/1. We interpreted this to mean that in the control animal a high rate of glycolysis supplies lactate as an energy source for the metabolic cycle. During hibernation glycogen storage takes place and during arousal all compounds show a decrease as a result of supplying energy for awakening.

Phosphate values were low for all fractions in both control and experimental animals. Either the amounts used for metabolic conversions are low or the values represent the composition of the rich vascular network of the brown fat.

Ultrasound. Gersten and Kawashima (1954), by means of ultrasound, produced an increase in PC and a decrease in IP without any rise in temperature in an isolated frog gastrocnemius. In our laboratory we applied clinical dosages of ultrasound to hind legs of control and hibernating ground squirrels. One of our more interesting findings (Zimny and Head in MS)

is that by means of ultrasound the phosphate fractions, IP, ATP, and PC, in the hibernating animal can be brought to arousal levels in a relatively short period of time without any change in the animal's rectal temperature.

Conclusion

Adenosine triphosphate is the immediate source of energy for metabolic work involving phosphorylations, syntheses, and other possible processes utilizing chemical energy. Phosphocreatine, glycolysis, and biological oxidations can effect a steady resynthesis of this compound. During hibernation, metabolic work is greatly reduced; the animal is stationary; the heart rate is greatly decreased; and digestive processes involving the liver are lessened. Cardiac muscle glycogen accumulates at the expense of skeletal muscle and liver glycogen and glyconeogenesis, primarily from fat, feeds metabolites slowly into the glycolytic system. Upon arousal adenosine triphosphate is used as the primary energy source with the high-energy phosphate of phosphocreatine maintaining adenosine triphosphate, and these combined reactions stimulating the energy producing cycle of glycolysis for the purposes of resynthesis.

Studies of these phosphate compounds in tissue extracts have been of value for interpreting metabolic adjustments but studies must now be extended to cellular and ultracellular structures. Enzymatic phosphate transfers by kinases, phosphatases and phosphorylases have prompted us to begin studies on the concentration of ATPase, ATP-creatine transphosphorylase and cholinesterase in the hibernating ground squirrel. The localization of oxidative phosphorylations in the mitochondria of muscle stimulates interest in possible structural changes of mitochondria in cardiac and skeletal muscle occurring during hibernation. Adenosine triphosphate is necessary for the functional integrity of the contractile elements of muscle, actin and myosin, and for the proper sarcoplasmic environment to act as a substrate for the myofibrils. Anabolic processes of cellular metabolism involving oxidations, CO_2 fixation, syntheses and secretory processes of the cell are related to adenosine triphosphate and the mitochondrial system.

The hibernant is capable of both lowering and raising its body temperature in a cold environment. Tissue phosphate compounds in a hibernating animal can be stimulated by the mechanical

means of ultrasound to assume levels comparable to arousal without an increase in body temperature. A rise in heart rate often precedes the rise in body temperature, but the converse is not true.

Extended investigation into the fields of enzymatic energy transfer mechanisms as related to ultracellular structure may not change any specific conversion we now know in the general scheme of intermediary metabolism in the hibernator but may show a heart, rich in mitochondria, possessing great oxidative capacity for the production of ATP, used for immediate energy when needed and resynthesized by a high-energy phosphate transfer from PC to ADP. A similar energy cycle may exist in skeletal muscle with the possibility of ATP production being more dependent on glycolytic processes of anaerobic metabolism than oxidative phosphorylation. In either case it appears that the energy change in the heart is the last link in the chain of metabolic events when the animal enters hibernation and the first spark during arousal.

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DISCUSSION FOLLOWING ZIMNY'S PAPER

FISHER asked what the body temperature was after 30 minutes of arousal. ZIMNY replied that it was between 16 and 17°C, a rise of about 9°C from hibernating body temperatures. POPOVIC asked where the thermocouple was placed. ZIMNY replied that it was in the rectum. FISHER noted that the core may be at a higher temperature than this. ZIMNY added that she expects to take temperatures at a higher level and analyze muscle samples from the forelimbs in the future.

BULLARD asked if she had noted the heart rate at the 30 minute point of arousal. She replied that it was about 190 beats per minute, an increase of about 174 beats from the hibernating heart rate.

JOHANSSON stated that he had also made lactate determinations in the heart muscle of the hedgehog, comparing hibernating and non-hibernating states. He found no significant difference between the two groups. ZIMNY said she used the Miller and Muntz method with a frozen tissue sample (B. F. Miller and J. A. Muntz, J. Biol. Chem., **126**:413, 1938). The heart must be removed very quickly before arousal has a chance to proceed. If it is not frozen immediately, some lactate may be lost; in fact, in phosphate determinations, all may be lost if the tissue is not handled very quickly.

BULLARD asked how changes in ATP and glycogen in the heart compared with other species. ZIMNY said she knew of no particular work on phosphates in other small animals that was comparable.

FISHER remarked that the production of large quantities of lactate is a common physiological method of getting a lot of energy in a hurry. ZIMNY agreed that lactate is probably useful for fast energy needs. She had not done blood work on this. With respect to high energy phosphates, they are extremely labile and can be lost just by putting a needle into the heart.

WIMSATT noted that glycogen lability in the liver also shows short-term deviations of concentrations. He stated that a student of his had found a definite decline in total liver glycogen in bats occurring over the spread of the hibernating season, with a substantial drop in the latter part of the season.

Concerning ZIMNY'S high liver ATP values for the ground squirrel, DENYES noted that liver slices from hibernating hamsters have a high oxygen consumption which is increased by the addition of succinate but not by glucose. This also indicates a retention of ATP during hibernation.

MUSACCHIA remarked that (adding to WIMSATT'S comments) there is a slow and steady utilization of liver glycogen in the hibernating turtle in a 4-6 week period, with utilization at a steady rate even though there is no maintenance of a steady body temperature.

XXV

SOME METABOLIC SPECIALIZATIONS IN TISSUES OF HIBERNATING MAMMALS[1]

By Frank E. South
Department of Physiology
University of Illinois College of Medicine
Chicago, Illinois

It has long been realized that the hearts, peripheral nerves and reflexes, as well as other tissues and organs, of hibernators are physiologically modified to the extent that they are capable of continued function at temperatures considerably lower than those of other mammals (cf. Hegnauer *et al.*, 1950; Lyman and Chatfield, 1955; Dawe and Morrison, 1955; McQueen, 1956). The problem, in paraphrase, is: what general or specific factor makes it possible for the heart of one animal, a hibernator, to beat at a temperature of 4°C while that of another animal will go into asystole or fibrillation at 15° or 20°C? The same question may be asked in regard to certain reflex patterns (e.g. respiration), nerve conduction and other processes.

Possible answers may range from simple enzyme or substrate concentration differences to some of the more abstruse concepts of kinetics and thermodynamics. Indeed, in a recent challenging paper Brown (1956) maintained that the hibernator could survive at low body temperature due to a higher setting of the reaction rates of its cellular processes relative to that of the non-hibernator. This means that while a given rate process common to both types of animals would have identical Q_{10}'s, the rate observed for the hibernator would be higher throughout the significant temperature range. This is one theory, and it does fit with some, but not all, of the facts.

Many biologists, including those interested in hibernation, have taken a clue from Crozier (1924) and attempted to analyze their data on the basis of either Q_{10} or the Arrhenius equation, one form of which is:

$$Ea = R\frac{\ln k_{T_2} - \ln k_{T_1}}{1/T_1 - 1/T_2}$$

or

$$k = Ce^{\frac{-Ea}{RT}}$$

[1] This work was supported by a contract with the Office of Naval Research, Contract No. Nonr-1459-05, and by a grant from the National Institutes of Health (H-5305).

in which k_{T_1} and k_{T_2} refer to the velocity constants at absolute temperatures, T_1 and T_2; R is equal to the gas constant and Ea is taken as the apparent "energy of activation" (μ is also a common symbol employed here).[2]

While such plots of the logarithm of the velocity against the reciprocal of the absolute temperature often yield straight lines over a limited range, especially for pure systems, they are more often curved lines for more complex systems such as heart rates. Such non-linear plots have been analyzed by assuming linearity over a limited portion, as was done by Crozier or by the empirical method of Kavanaugh (Kavanaugh, 1950; South, 1958). Although certain of the assumptions of both of these approaches have been questioned on theoretical grounds, yet when used with caution and with properly controlled experiments, such analyses may prove to be extremely powerful tools in the exploration of the basic phenomena of hibernation.

A rational basis for utilizing temperature analyses is provided by comparing temperature plots of operationally identical systems (e.g., rates of tissue oxygen consumptions) of hibernating and non-hibernating animals. By progressive dissection, from the complex to the simpler, it should become possible to define those factors upon which the hibernant depends for the maintenance of life at low temperatures.

This principle may be illustrated by data from studies which have been made upon cardiac muscle and upon peripheral nerves and skeletal muscle.

The first thorough study of this type upon the comparative effects of temperature upon neural conductivity was that of Chatfield *et al.* (1948). Using the tibial nerves of golden hamsters and rats they were able to show that hamster nerves could conduct a spike potential at much lower temperatures than could those of the rat (means at which conduction ceased were 3.4° and 9°C, respectively); the range of incubation temperatures was 2° to 20°C. Of equal significance was the observation that with descending temperatures the action potential, excitability and conduction velocity of rat nerves declined as a steep function. In contrast, the conduction velocity and excitability of hamster nerves fell off much less with lower temperatures

[2] It is generally agreed that the experimental energy of activation is that energy required by the reactants to reach an intermediate complex or configuration which is necessary in order that the reaction may proceed to completion. According to the most prominent view, enzymes affect reaction rates by reducing the energy barrier (i.e. the energy of activation) over which the reactants must pass in order to form products (Glasstone *et al.*, 1941).

while the action potential passed through a maximum at 15°C, declined rather slowly to 5°C, and rapidly thereafter. No differences between hibernating and non-hibernating hamsters were observed.

In a series of experiments, in which phrenic nerve-diaphragm preparations obtained from rats and from both hibernating and non-hibernating hamsters were studied, it was found that the results obtained from the phrenic nerves were qualitatively quite similar to those of the experiments cited above. One fundamental difference did appear, however, in that the excitability at 5°C was greater in the case of the hibernating hamsters than for hamsters maintained at room temperature (South, MS in prep.). The use of the phrenic nerve-diaphragm preparations proved to be extremely useful in that they provided an opportunity to study a number of the properties of both nerves and muscles in relationship to temperature, hibernation, and the possibility of phylogenetic adaptation. Some of the results will be discussed below.

One of the more interesting results which were obtained related to myoneural transmission. It was observed that while all of the rat phrenic nerves were capable of conducting a spike at a temperature of 10°C there was no recordable isometric muscle response in 70 per cent of the instances. At 5°C the rat phrenic nerves were inexcitable but those of the control and hibernating hamsters retained their ability to conduct spikes. However, in no case was there evident muscular response to indirect stimulation on the part of preparations obtained from control hamsters, although those obtained from hibernating hamsters always responded to such stimuli at 5°C. That these observations were dependent upon the functioning of the myoneural junction could be shown by the ability of the muscles of all three groups to respond to direct stimulation, although the necessary stimulus varied widely, and by appropriate tests with tubocurarine. These observations are now being subjected to further study.

Considering the differences in irritability of peripheral nerves, which have been summarized above, it is not surprising that an analogous situation should be found to hold true for diaphragm muscle. The threshold for direct stimulation of rat muscles rose to relatively high values below 17°C. The hamster thresholds rose to a lesser degree and were quite similar to one another down to 10°C. Below this point the threshold for the muscles of control hamsters became very high relative to that of diaphragm obtained from hibernating hamsters.

The contractile responses of the diaphragms were measured isometrically, the muscles being stimulated at rest length. The rates of tetanic contraction and relaxation were treated by plotting the reciprocal of the "half-time" for either process against the reciprocal of the absolute temperature (Figs. 1, 2).

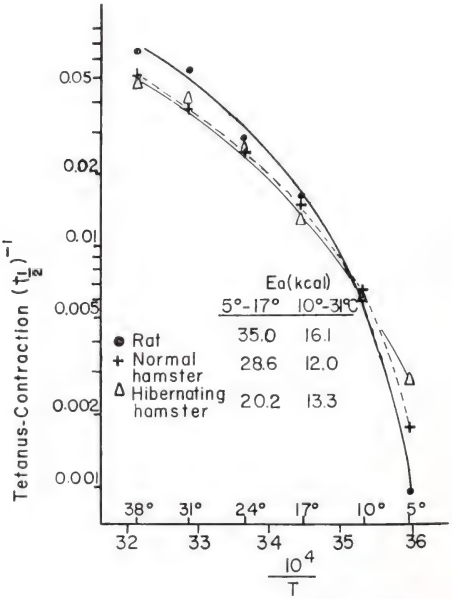

Fig. 1. Relationship between isometric contraction rate of diaphragm muscle, *in vitro*, and temperature. Semilogarithmic plot. Each point represents an average value of 6 to 10 separate experiments. pH = 7.4.

The use of the half-time under these conditions is based on the treatment of Ramsey (1944), whose equation follows first-order kinetics and describes the rate of tension production from rest length with fair precision. Therefore the reciprocal of the contraction half-time would be proportional to the specific first-order rate constant. Such a treatment is sufficient for our purposes.

Proceeding on these assumptions, it is immediately apparent that the rate of rat muscle tension production is more temperature dependent than that of hamsters (Fig. 1). Straight lines fitted to any portion of the curve (e.g. the fairly linear range, 10°-31°C) indicate a higher Ea for the rat than for the hamsters.

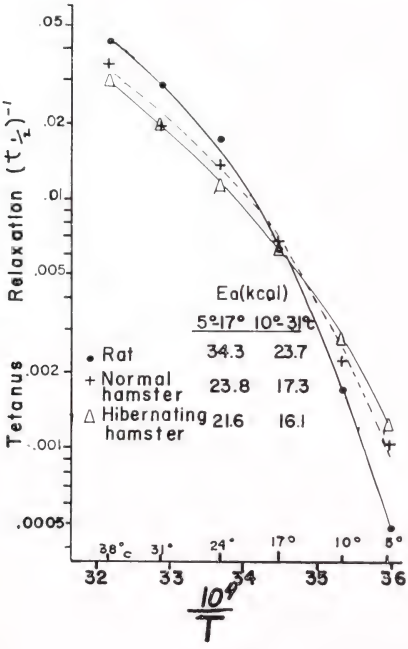

Fig. 2. Relationship between relaxation rate from isometric tetanus, *in vitro*, and temperature. Semilogarithmic plot. Each point represents an average value of 6 to 10 separate experiments. pH = 7.4.

At 5°C the rate of contraction is less than one-third that of the hibernating animal. It is also noteworthy that although the curves obtained from the hamsters lie very close to each other within the range of 10°-38°, they become divergent below 10°C. This result mimics to some extent the irritability differences noted above.

Plotting the rate of relaxation from tetanus (Fig. 2) reveals similar comparative results for the rat relative to the hamster groups, the difference being especially marked in the low temperature range (5-17°C). It also may be noted in Figure 2 that there is a fairly good indication of a lower value of Ea for the data obtained from hibernating hamsters as compared to their controls. The differences in these curves could be made more dramatic by plotting the data of the ordinate as per cent of maximum.

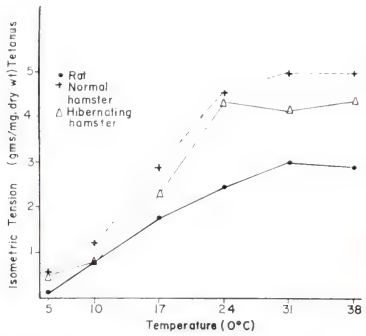

Fig. 3. Isometric tension production as a function of temperature. Rectangular coordinates. pH = 7.4.

While the application of first-order reaction kinetics to the relaxation period can be questioned on theoretical grounds, this treatment does serve to illustrate the relative temperature dependencies.

The relationships between tension produced by isometric tetanus and temperature (Fig. 3) in the three groups of animals reveal the rather surprising result that the control hamster diaphragms were capable of the greatest tension production, followed by the hibernating hamster and finally the rat. In this instance the rat muscle differs from that of the hamster not only in the lower tension production but also in a somewhat slower decline in tension with temperature. Arguing teleologically, one might be led to expect that the tension production by hamsters would be less affected by temperature than is the case in the rat. This is clearly not so.

The general pattern which emerges for nerve and muscle preparations indicates a fundamental functional dichotomy between hibernating and non-hibernating mammals, insofar as the rat and the hamster are representative of these groups. Hence, in these systems, other than tension production, the rat has a higher "setting" at 38°C but due to the greater temperature dependence (higher Ea's) the processes are slower and less effective in the low temperature range than is the case for hibernants. This dichotomy may be characteristic of those metabolic and functional systems which must be specifically and phylogenetically adapted for survival at low body temperatures.

It was noted above that frequently the temperature curves for certain processes of hibernating and control hamsters were very similar (e.g., magnitude of the spike potential and rate of conduction) throughout the temperature range. In other instances (e.g., threshold and contraction rate) they were very close down to a temperature *ca* 10°, whereupon a divergence occurred in which the curve for the control hamster fell off more sharply than that for the hibernating hamster. The plots of the relaxation rates revealed the possibility of yet another factor, that is, a difference between hibernating and non-hibernating hamsters similar to that between rats and hamsters. Such observations led one to suspect that something more than mere interspecific differences are involved here. In other words, the hamster may undergo an acclimatization process prior to hibernation which involves cellular processes at a very fundamental level.

It is not enough, however, that only the kinetics of muscular contraction or neural conduction be adjusted for function at reduced temperatures. These are only mechanical or physical-chemical results of many metabolic processes which function to "serve up" energy in sufficient quantities and in usable form (e.g. ATP). Consequently, any self-contained system which is critical for survival during hibernation must be modified in three ways with respect to temperature. That is, it must be able to (1) transform the energy content of general substrates (e.g. glucose) to an immediately useful form (e.g. ATP) at a sufficient rate to support the needs of the overall process. (2) Intermediate coupling (e.g. ATP to myosin) must be accomplished at a sufficient rate. (3) The "effector" portion of the system (e.g. contractile protein, metabolic "pumps," etc.) must be able to transfer this energy to its environment at a sufficient rate and quantity to meet the needs of other systems. Therefore, a hypothetical tissue might supply energy at a high rate, but it would be of no consequence if the effector should be unable to use it.

The heart represents a system which has received a fair amount of attention from those interested in mammalian hibernation. We are well aware of its central position of importance in hibernation, functioning not only as the circulatory pump but also as an important source of heat during arousal (Lyman and Chatfield, 1950; Lyman and Leduc, 1953). A number of investigators have examined heart rates and EKG's of hibernating, awakening and non-hibernating mammals (cf. Kayser, 1953; Lyman and Chatfield, 1955, for discussion and bibliography). One particularly interesting aspect of the EKG studies relates to the repolarization time (S-T). Since repolarization time is fairly long it dominates the intervals variously reported as Q-T and RS-T. Evidence indicates that of all intervals of the EKG, the Q-T is most profoundly affected as the heart temperature of non-hibernators declines and aberrancies of the S-T segment (Osborn wave) often occur (Nardone *et al.*, 1955; Ruhe and Horn, 1955; Biörck and Johansson, 1955; Tysinger *et al.*, 1956). In contrast, the repolarization time of hibernators is usually less affected by declining temperatures than the other intervals and seldom shows aberrancies of the type described by Osborn (1953) (cf. Dawe and Morrison, 1955; Biörck and Johansson, 1955; Nardone, 1955). Since repolarization is dependent upon metabolic processes (Garb and Chenowith, 1953), it is quite apparent that the comparatively short S-T interval seen for the hearts of hibernating mammals represents the resultant of phylogenetic adaptation of cardiac metabolism.

The question remains as to modifications in the mechanisms of energy supply. Several solutions are possible: (1) the inherent rate of supply might be sufficient so that only the "effector" is modified; (2) the inherent rate may not be sufficient, but increases in the concentrations of rate-limiting enzymes and primary substrates might occur such that enough energy could be supplied to the effector (alternate pathways would also be included here); or (3) the *properties* of the enzyme system, or of a rate-limiting enzyme within that system, might be altered in such a way that at lower temperatures it would operate more efficiently in reducing the "energy barrier" over which the reactants must pass.

As a first step in answering these questions, the initial rates of oxygen consumption of heart slices obtained from rats, control hamsters, hibernating hamsters, and torpid bats (*M. lucifugus*) were determined over a temperature range of 5° to 43°C (Fig. 4) (South, 1958). Again the now familiar effect of temperature on

rat tissue is seen — the rapid fall of rate from an initially high value. In a fashion similar to that observed for the rate of relaxation of diaphragm muscle (Fig. 2), the ventricular QO_2 of the control hamsters declines less rapidly than for the rat and more rapidly than for the hibernating hamster and bat. If one "force fits" a straight line to the curves between the temperatures of 17° and 43°C for comparative purposes, the calculated Ea's for the rat, control hamster, hibernating hamster and torpid bat, respectively, are 9.8, 6.0, 4.8 and 4.6 kcal. It may be concluded that in certain systems the hamster does "prepare" for the hibernal situation on a cellular level. From all evidence

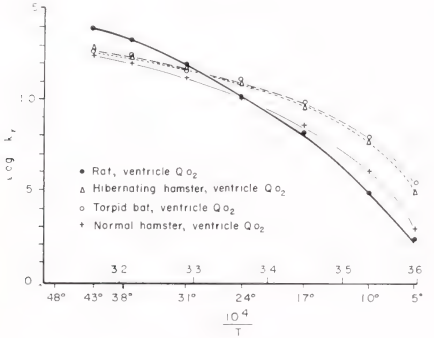

Fig. 4. Logarithm of the initial QO_2 of ventricle slices (k_r) as a function of the reciprocal of the absolute temperature of incubation.

presented so far, this is not accomplished by merely increasing concentrations of enzymes and by storing substrates as would be indicated if the rate-temperature plots were parallel with different intercepts. On an operational level, the lower Ea's for the hibernators suggest an adaptation of enzyme properties. Whether this means that the curves which have been obtained are due to a metabolic shift such that they are characteristic of different limiting enzymes, alternate pathways, or to adaptations of the properties of given enzymes which make them less susceptible to changes in configuration over a range of temperature (as might be suggested by Kavanaugh's hypothesis (1950)), is not at all certain.

That these differences in rate temperature plots are probably not ubiquitous among the tissues was shown by similar experiments with brain slices. In this case, the curves for brain QO_2 were essentially parallel with only slight differences in potential intercepts (Fig. 5). The Ea's were approximately 11.0 kcal. except that for bat brain, which was slightly lower at 10 kcal.

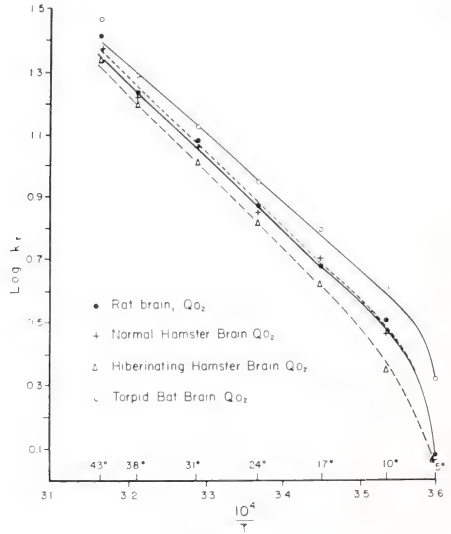

Fig. 5. Logarithm of the initial QO_2 of brain slices (k_r) as a function of the reciprocal of the absolute temperature of incubation.

Similar results were obtained for the rates of anaerobic glycolysis of brain slices, with a slight suggestion of lower Ea's for hibernating animals (South, 1958). This result was rather unexpected in view of the experiments of Peiss and Field (1950) in which brain minces of the Arctic Cod and Golden Orfe were studied. These animals lived at ambient temperatures of $-1.5°$ to $+2.0°C$, and $+25°C$, respectively. The Ea values between 10° and 25°C, as calculated from their published data, were 12.3 kcal for the Arctic Cod and 16.0 kcal for the Golden Orfe. Between 10° and

0°C the Ea for the former fish did not change significantly while that of the Orfe rose to about 25 kcal — a pronounced decrement in rate.

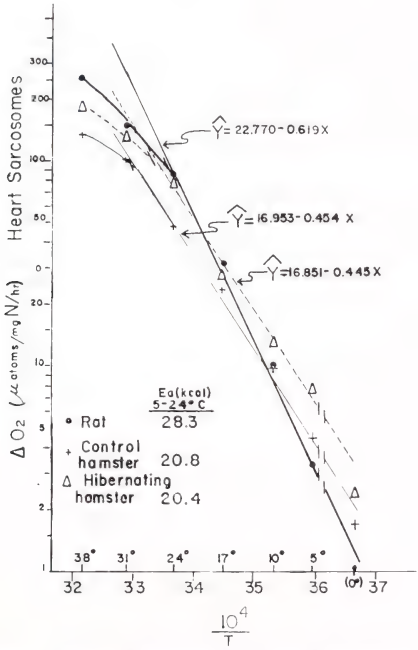

Fig. 6. Rate of oxygen consumption by heart mitochondria as a function of the reciprocal of the absolute temperature of incubation. Semilogarithmic plot. Each point represents the mean of 6 to 10 experiments. Pyruvate (malate) substrate.

Just as not all tissues or systems evidence the same apparent adaptive response of cardiac tissue, so it is to be expected that not all the reaction of energy metabolism will mirror these alterations. This implies that only those enzymatically-controlled reactions which may be rate-limiting under these conditions need be altered. Furthermore, the phylogenetic adaptation which discriminates between the hamster which can hibernate and the rat

which cannot may occur at a different locus in the metabolic chain than does the change which apparently distinguishes the hibernating hamster at 5°C from the hamster dwelling at a comfortable 25°C. It must be confessed that these statements were made utilizing the peculiar prognostic powers obtained from a finished experiment.

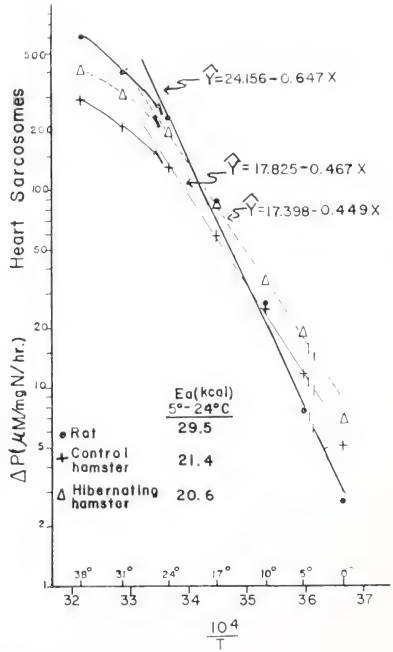

Fig. 7. Rate of esterification of inorganic phosphate by heart mitochondria as a function of the reciprocal of the absolute temperature of incubation. Semilogarithmic plot. Each point represents the mean of 6 to 10 experiments. Pyruvate (malate) substrate.

As part of the continuing effort to characterize and isolate those metabolic factors involved in hibernation, heart mitochondria of rats, control hamsters, and hibernating hamsters were

isolated. The rates of oxygen uptake and phosphate esterification and "P/O ratios" were determined (cf. Maley and Plaut, 1953, for general methodology, which was modified for use here). Pyruvate, with malate as a "sparker," was the substrate. Incubation temperatures ranged from 0°C to 38°C.

Reference to Figure 6 reveals that even at the mitochondrial level the rat heart remains very different, the slope of the line of rate of oxygen uptake is again steeper and crosses those of the hamsters as before. An additional fact is also immediately apparent in that the slopes of the rates of oxygen consumption by hibernating and control hamsters are parallel. This can be interpreted only as an increased enzyme concentration, accompanying the hibernating state, at some point along the portion of

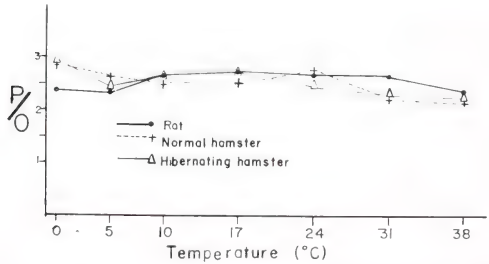

Fig. 8. P/O ratio of heart mitochondria as a function of temperature. Rectangular coordinates. Pyruvate (malate) substrate.

the metabolic pathway responsible for oxidative metabolism of pyruvate. These observations are reinforced by the almost identical graphic form of the rate of phosphate uptake (Fig. 7) and the closeness of the values of Ea. These plots and that of Figure 8 indicate that while the *efficiency* of phosphorylation is essentially unaffected by temperature and not related to hibernation as such, the *rate* at which it is accomplished is of central importance.

It appears, then, that a specific enzymologic difference exists between rats and hamsters along the pyruvic oxidase, dehydrogenase and terminal electron acceptor systems. Precisely where this lies, it is not yet possible to say. The factors responsible for

the change in slope correlated with the assumption of hibernation by hamsters (Fig. 4) have been dissected out of the oxidative phosphorylation system as studied here. The change in concentration is, in all probability, an ancillary change.

Before proceeding, it would be well to emphasize that while the various values of Ea have been diligently calculated, direct comparison with values obtained in other experiments and by other authors studying systems of various complexities has been avoided. For those desiring such data, the articles of Sizer (1943), Kavanaugh (1950), Johnson *et al.* (1954), and Feigen *et al.* (1958) may be consulted.

The reasons for this avoidance are several. Too many variables exist, such as pH, ionic milieu and strength, the question of possible inactivation at temperature extremes, presence of various inhibitors, and the subjective factor of just how and where the curves or slopes are fitted. Unless it can be shown that a particular catenary series of reactions depends upon a specific rate-limiting reaction as characterized by the Ea it very well may be misleading to assume such a relationship. An example of this might be to conclude, since the rate of contraction of diaphragm muscle of hibernating hamsters has an Ea = 20 kcal and bacterial dehydrogenase has an Ea = 19.4 kcal, that the rate of contraction of the muscle is limited by the bacterial dehydrogenase reaction. Such a procedure must be approached with caution.

Since the approach of listing various reactions and comparing Ea's has been rejected, we are left with the problem of explaining why, in so many systems, the Arrhenius plots are less steep for hamsters than for rats, and for hibernating than for control hamsters. Several possibilities, some of which have been discussed above, can be cited, but few definitive answers given.

In view of the slower reaction rates at higher temperatures for hibernants relative to the non-hibernants, and the crossing over to a comparatively high rate as the temperature declines, the suggestion of Brown (1956) may be dismissed as a generic solution. It does have some application as exemplified in the rates of oxidative phosphorylation of heart sarcosomes of hibernating and control hamsters and, possibly, to isometric tension production as well (Fig. 3).

All other explanations must remain in the limbo of hypotheses until the crucial experiments have been completed. However, certain of these possible explanations have an attractiveness which may be of value in the design of experiments.

The easiest explanation would involve the idea that these curves do represent the energies of activation of limiting reactions in a catenary series. Hence, the hibernator would have adapted by, again, increasing the concentration of a given enzyme to the degree that it no longer would be limiting and, therefore, the curves represent the Ea of a different enzyme. If this were so, one would expect that the rate of the reaction at higher temperatures would be greater than that for the same reaction of the rats, regardless of the slopes. This does not occur. A similar objection would apply to most direct applications of Crozier's (1924) theory, although it could obtain in certain circumstances.

In an attempt to explain the lack of linearity of Arrhenius plots and the alterations in rates at temperature extremes, Kavanaugh (1950) developed an hypothesis which considered changes in configurations of the enzymes. Hence, at higher temperatures reversible denaturation of the enzyme occurs with a concomitant reduction in its specific configuration (cf. Johnson *et al.*, 1954). In this partially "unfolded" or "uncoiled" state the "active centers" would no longer be in proper alignment for maximal activity. At low temperatures, the formation of additional intramolecular bridges would result in the opposite situation, that is, the enzyme would be excessively "folded" so that the "active centers" would again be out of optimal alignment. By implication, it was argued that a single reaction *could* be limiting for a complex system throughout the temperature range. Such a formation could be adapted for our purposes by postulating that certain critical enzymes of the hibernator might be modified through formation of "stabilizing bonds" in a way that such deviations from an intermediate configuration would be minimized. Such an hypothesis is not inconsistent with the results as reported here except that it also implies that all Arrhenius plots of enzymatically controlled reactions must be curved, *a priori*.

On a rather intuitive basis, a more attractive formulation might be constructed from the experiments of Fraser and Kaplan (1955) on yeast catalase. They found evidence, on the basis of their values for Ea and other thermodynamic constants, that intracellular yeast catalase is adsorbed to an interface in a partially unfolded configuration of low specificity and with a high $\Delta H^{\ddagger}$ of activation. Destruction of this interfacial association of the catalase by various agents decreased the $\Delta H^{\ddagger}$ to that seen for the reaction when catalyzed by extracted yeast and

crystalline liver catalases, i.e. from an original level of 8.5 kcal/mole to about 4 kcal. Applying the interfacial hypothesis to hibernation it could be postulated that while the enzymes of the rat usually exist in an analagous association with intracellular interfaces and structures with a concomitant loss of ordering and high Ea's, the critical enzymes of hibernators exist in less unfolded, less restrained but more active configurations. The processes catalyzed by them would possess lower energies of action.

While at the present time little additional evidence can be brought forth to support this idea, it is receiving considerable attention in our laboratory at the present time.

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DISCUSSION FOLLOWING SOUTH'S PAPER

STRUMWASSER noted that, in frogs, neuromuscular functioning continued at low temperatures. Choh-Luh Li and P. Gouras (Am. J. Physiol., **192**:464, 1958) showed that spontaneous miniature end plate potentials (recorded intracellularly from the sartorius muscle) and contraction still occurred as low as −1°C in response to direct and indirect electrical stimulation. He then asked, concerning SOUTH'S experiments on differences in neuromuscular transmission between hibernating and non-hibernating mammalian species, whether his *in vitro* experiments had taken into account natural intercellular parameters which may be the important factor in the hibernating animal rather than some fundamental difference in muscle or nerve. SOUTH replied that he knew of Choh-Luh Li's paper, but he could only say that the results described were as he (SOUTH) obtained them. He indicated he intended to carry the work further using micro-electrodes.

STRUMWASSER then asked if, using the proper frequency, duration and waveform of electrical stimulus to the nerve terminals at the neuromuscular junction, one might not be able to extend neuromuscular transmission in the rat to lower temperatures than SOUTH had described. SOUTH replied that he used various frequencies, strengths and durations, and no differences were obtained. The optima were reported.

XXVI

THE EFFECTS OF IONIZING RADIATION IN HIBERNATION[1]

By Douglas E. Smith
Division of Biological and Medical Research
Argonne National Laboratory
Lemont, Illinois

The subject matter of this paper falls into two broad categories. One concerns the use of hibernating animals as tools in the study of the development of and protection against damage by ionizing radiations. The other deals with the effects of ionizing radiations on the induction and maintenance of the state of hibernation.

I. The Development of Radiation Damage in the Hibernating Mammal

A. *Introduction.* Practically nothing is known of the sequence of events that lead up to the morphological and biochemical lesions detected hours and days after the irradiation of mammals. Because of its markedly low body temperature and metabolism, the hibernating mammal appears to be a promising tool for the study of the development of radiation damage. Although one would not expect the changes attending the initial transfer of energy from incident radiation to be influenced within the range of temperatures found in homeothermic and hibernating animals, one would anticipate that the subsequent chemical changes would be temperature-dependent. It is possible in hibernating animals not only that the development of radiation damage might be generally slowed but also that it might be different when compared with that in irradiated homeotherms. Such differences might be expected on the basis of the complex interrelationship of possible alternate metabolic pathways, differences in activation energy of enzymes and different radiosensitivities of enzymes.

To the present, two main types of study have been carried out on the effects of ionizing radiations on hibernating mammals. In one, account has been taken of the mortality of x-irradiated hibernating animals. In the other, histopathological examinations have been made on the tissues of hibernators similarly

[1] This work was performed under the auspices of the U. S. Atomic Energy Commission.

treated. Observations have been made also on a few other signs of radiation injury.

B. Lethal effects. Table I summarizes the mortality data gathered on several species x-irradiated while hibernating and kept in hibernation for varying periods of time thereafter. It is clear from these data that the expression of radiation damage as mortality is markedly slowed during hibernation. In the ground squirrel and dormouse there is no evidence for development of damage resulting in death during the period of hibernation, whereas the data from bats clearly indicate the development of such injury in the hibernating state. (Multiple hemorrhages and coagulated blood, gross signs of radiation damage, were found in the gastrointestinal tract of bats dying during the period of hibernation after irradiation (Osborn and Kimeldorf, 1957). In bats it is also obvious that the level of mortality during hibernation is dependent upon radiation dosage. Furthermore, in the little brown bat observations beyond the 30-day period shown in Table I reveal a progressive development of damage leading to death (cf. the 15,000 r group of Figure 1).

If, after the hibernation periods shown in Table I, the animals are removed to and kept in a warm environment, death occurs as in the case of non-hibernating animals irradiated and kept in the warm environment. According to some workers (Künkel *et al.*, 1957) the mortality pattern is that to be expected if the animals had not been exposed to x-rays until brought into the warm environment. Examination of the available detailed mortality data, however, reveals that for ground squirrels (Smith, 1959a) and marmots (Smith and Grenan, 1951a) the time to 50 per cent mortality and the average survival time (calculated from the time of removal to the warmer room) are shorter in the groups exposed to radiation while hibernating than in the groups exposed and kept in the warm environment. This indicates that radiation damage leading to mortality is developed during the period of hibernation. Quite the opposite results are found in the dormouse (Künkel *et al.*, 1957). Here the average survival time and time to 50 per cent mortality calculated after removal from hibernation is about 3 times as long in the group irradiated while hibernating as in the group irradiated and kept at 20°C. Moreover, the mortality at 30 days in the 20°C environment is 67 per cent in the former and 83 per cent in the latter group. These data indicate the possibility that the 21-day period of hibernation after exposure to x-rays confers some protection against damage leading to mortality.

TABLE I

Mortality of X-irradiated Mammals During Hibernation

Treatment	Reference	No. of animals	No. of days in hibernation after irradiation	% mortality
Ground squirrel (*Citellus tridecemlineatus*)				
800 r	(1)**	24	30	0
1000 r	(1)	12	30	0
1200 r	(1)	12	30	0
1000 r	(2)	90	22	0
2000 r	(2)	4	21	0
Control*	(1,2)	60	—	80-100
Marmot (*Marmota monax*)				
650 r	(3)	7	28-42	14
800 r	(3)	1	21	0
Control*	(3)	9	—	67
Dormouse (*Glis glis*)				
700 r	(4)	21	21	0
Control*	(4)	18	—	83
Pallid bat (*Antrozous pallidus pacificus*)				
1500 r	(5)	9	42	56
3000 r	(5)	21	42	43
6000 r	(5)	9	42	89
Control*	(5)	35	—	100
Yuma bat (*Myotis yumanensis saturatus*)				
500 r	(5)	8	42	13
Control*	(5)	10	—	80
Little brown bat (*Myotis lucifugus*)				
500 r	(6)	40	30	30
1000 r	(6)	40	30	25
5000 r	(6)	40	30	32
15000 r	(6)	40	30	52
Control*	(6)	160	—	100

* The controls were irradiated and kept at 20-27°C. The mortalities are for the same irradiation dosages and times after exposure as in the hibernating groups.

** (1) Doull and DuBois (1953); (2) Smith (1959a); (3) Smith and Grenan (1951a); (4) Künkel *et al.* (1957); (5) Osborn and Kimeldorf (1957); (6) Smith (1959b).

C. Tissue damage. Histopathological findings have been reported for ground squirrels (Fitch *et al.*, 1955) and marmots (Brace, 1952) during hibernation, after exposure to x-rays. Evidence of distinct cellular damage (failure of mitosis, nuclear changes and cell death) was found in the spleen, lymph nodes and bone marrow of the hibernating ground squirrel as early as 6 hours after a dosage of 800 r. Thereafter the rate of development of cellular damage was substantially the same in the hibernating and non-hibernating animal. Preliminary studies (S. W. Lesher and D. E. Smith, unpublished observations) have shown somewhat similar results in the crypts of the duodenum of the hibernating ground squirrel exposed to 1000 r. In contrast to the findings in the ground squirrel, no signs of cellular damage were reported (Brace, 1952) for bone marrow, lymph nodes, spleen, lungs, heart and adrenals of irradiated hibernating marmots. Loss of ovocytes, ovogonia, spermatocytes and spermatogonia were observed. Signs of hematopoiesis were absent in both the irradiated and non-irradiated marmot throughout periods of hibernation as long as 8 weeks. Upon removing both groups to a warm environment, hematopoiesis was apparent after two days and high at 7 days in the non-irradiated animals. No recovery of hematopoiesis was noted in the irradiated group even after 7 days in the warm environment. In the ground squirrel, hematopoiesis was absent during the 29-day period of hibernation after irradiation and failed to return after three days in the warm room. This finding is in contrast to the observation of significant recovery of hematopoiesis at 3 days after irradiation of animals always in the warm environment. Here again is a possible difference between hibernating and non-hibernating animal with respect to the effects of radiation damage. Thus, not only does it appear that recovery of hematopoiesis is not possible during hibernation after irradiation but it also seems that hibernation was attended by changes that do not allow the return of hematopoiesis when the ground squirrels are removed to a warm environment.

D. Peripheral blood. During 42 days of hibernation after exposure to 800 r the marmot is reported (Smith and Grenan, 1951b) to show no changes in the number of circulating red or white blood cells or in the pattern of the differential blood count. Upon removal to a warm environment the levels of both the red and white blood cells fell rapidly. No change in circulating blood cells has been found (Künkel and Schubert, 1959) in the dormouse. Indirect indications that the same is true for the ground

squirrel (Fitch *et al.*, 1955) come from the finding of increased hemosiderin in the red pulp of the spleen in irradiated, non-hibernating, but not in the irradiated, hibernating animal. The above data suggest that the life-span of the blood cells is markedly prolonged in the hibernating state.

E. Chemical measurements. Alkaline phosphatase activity in the spleen of the ground squirrel is increased after irradiation, but the increase is smaller in the hibernating than in the non-hibernating animal (Peterson and DuBois, 1952). Changes in serum proteins are significant in the irradiated, non-hibernating dormouse but are absent during hibernation after irradiation (Schubert *et al.*, 1957). The incorporation of P^{32} into deoxyribonucleic acid of the intestine in the irradiated, hibernating dormouse, however, is 50 per cent lower than that in its non-irradiated, hibernating control (Künkel and Schubert, 1959).

F. Conclusions. It seems clear from the existing data that lethality resulting from x-irradiation is markedly slowed in the hibernating mammal. Further conclusions must be made with considerable caution because of the limited and fragmentary nature of the experiments. A number of other influences of hibernation on the development of radiation damage are strongly suggested by the existing information, however, and should be mentioned. Thus, there appear to be species differences with respect to the development of cellular damage (cf. the ground squirrel and marmot) and to the development of damage leading to lethality (cf. posthibernation mortality of the ground squirrel and marmot in comparison with that of the dormouse). The latter is of especial interest, since it indicates that there is repair or failure of development of lethal damage in the irradiated, hibernating, as compared with the irradiated, non-hibernating dormouse. Hibernation appears to prevent or slow the recovery of at least one process inhibited by irradiation (cf. the absence of recovery of hematopoiesis of the irradiated, hibernating ground squirrel or marmot upon removal to a warm environment). It would seem highly worthwhile that the above phenomena be thoroughly investigated. It is possible that the explanation (based on preferential metabolic pathways, different activation energies of enzymes and different radiosensitivities of enzymes) suggested above can account for the qualitative differences between the hibernating and non-hibernating animal with respect to the expression of radiation damage. These differences suggest that when more detailed examinations of biochemical systems are made in studies of hibernation *per se*, phenomena

explained by differences in activation energy of enzymes and alternate metabolic pathways will be detected.

II. Influence of Hibernation upon Chemical Protection Against the Lethal Effects of Ionizing Radiations

Significant protection against the lethal effects of ionizing radiation is obtained in various homeothermic mammals when certain sulfhydryl compounds are administered before irradiation but not when such treatment is given after irradiation (Patt, 1953). Thus 80 to 100 per cent of rats injected with cysteine prior to irradiation will survive a 100 per cent lethal dosage of x-rays. No survivors are found when cysteine is administered after exposure. It has been postulated that the sulfhydryl compounds exert their protective effect during the act of irradiation by reacting with the decomposition products of irradiated water, by causing tissue anoxia, or by forming complexes with important constituents of tissue. It is of great interest, therefore, that marked protection by cysteine has been reported (Künkel *et al.*, 1957) in the dormouse irradiated while hibernating at 4°C but not treated with the sulfhydryl compound until 3 weeks after irradiation when the animals were removed to a warm room. The details of the experiments are presented in Table II. None of the irradiated animals died during the 3-week period in the cold. During the ensuing 30 days in the warm environment, however, 67 per cent of the non-cysteine-treated, irradiated dormice died; none of the animals injected with cysteine died during this time. In the same study, cysteine administered within 3 minutes after irradiation to the 12 non-hibernating dormice always kept in the 20°C environment was followed by 83 per cent mortality in the following 30 days. It thus appears that the mechanism of the protective action of cysteine may be the same in the non-hibernating dormouse as it is in the homeothermic mammal. In the case of protection by cysteine given 21 days after irradiation of the hibernating dormouse, however, an entirely different mechanism of action must be sought, since one would not expect temperatures as high as 4°C to prolong for 21 days the lifetime of the immediate products of irradiation postulated for the site of action of cysteine in the homeotherm. It seems possible that the low temperature of the hibernating dormouse may, because of different radiosensitivity of enzymes and different activation energies of enzymes leading to alternative metabolic pathways, bring about biochemical changes unlike those

encountered in the homeothermic animal in that they can be repaired by cysteine. In the absence of administration of cysteine, these biochemical changes are ultimately expressed as death.

TABLE II

Influence of Cysteine upon Mortality after X-irradiation

No. of animals	Treatment	% mortality during 30 days in warm room
	Dormouse (*Glis glis*) (Künkel *et al.*, 1957)	
21	700 r while hibernating at 4°C, transferred to 20°C after 21 days.*	67
15	700 r while hibernating at 4°C, transferred to 20°C after 21 days* and injected with cysteine, 500 mg/kg, i.p.	0
18	700 r while awake at 20°C.	83
10	Cysteine, 500 mg/kg, i.p. before 700 r while awake at 20°C.	0
	Ground squirrel (*Citellus tridecemlineatus*) (Smith, 1959 a, 1960)	
15	1000 r while hibernating at 5°C, transferred to 23°C after 21 days.*	87
15	1000 r while hibernating at 5°C, transferred to 23°C after 21 days* and injected with cysteine, 950 mg/kg, i.v.	80
20	1000 r while awake at 23°C.	80
20	Cysteine, 950 mg/kg, i.v. before 1000 r while awake at 23°C.	0
16	1000 r while hibernating at 5°C, transferred to 23°C after 15-30 min.*	81
16	1000 r while hibernating at 5°C, transferred to 23°C after 15-30 min. and injected with cysteine 950 mg/kg, i.v.	75

* No animals died during the 21 days or 15-30 minutes of hibernation after irradiation.

Protection by cysteine given after irradiation of the hibernating ground squirrel does not seem to occur (Table II). This is not surprising when one considers the histopathology of the irradiated ground squirrel (Fitch *et al.*, 1955). Cellular damage is evident in tissues of the ground squirrel as early as 6 hours after exposure to 800 r of x-rays and, as pointed out above, the

rate of development and the ultimate degree of damage thereafter is about the same in the hibernating and non-hibernating ground squirrel (Fitch *et al.*, 1955).

It appears that there is a distinct difference between the ground squirrel and the dormouse with respect to the possibility of protection by cysteine given after irradiation. Since no information is available concerning the histopathology of the irradiated, hibernating dormouse, no explanation involving differences in tissue damage can be attempted. One can only say that the development of radiation damage in the hibernating dormouse is different from that in the hibernating ground squirrel with respect to the existence in the dormouse of a biochemical defect that is reparable by cysteine. Such a defect is not present in the hibernating ground squirrel either at 21 days or one-half hour after irradiation.

III. The Influence of X-irradiation upon the Induction and Maintenance of Hibernation

To the knowledge of the author, there has been carried out but one set of experiments (Smith, 1959b) on the effects of radiation upon the induction and maintenance of hibernation. These experiments consist of exposing bats (*Myotis lucifugus*) and ground squirrels (*Citellus tridecemlineatus*) to various dosages of x-rays in the non-hibernating state (ambient temperature 23°C), placing them in a cold room at 5°C immediately after irradiation, and observing them for hibernation thereafter. Tables III and IV show that irradiation can prevent the induction and maintenance of hibernation in both species. It is apparent that massive dosages of x-rays are necessary for the effect. In the case of the bat, the phenomenon is dose-dependent, for as the amount of radiation is increased, fewer animals enter hibernation (Table III). Irradiated bats that fail to hibernate within the first 15 hours in the cold environment do not become dormant thereafter and die during the ensuing 33 hours (Fig. 1). On the other hand, bats exposed to 15,000 r, 30,000 r, 40,000 r, or 60,000 r and kept at 23°C survive as long as 15, 15, 7 and 4 days, respectively (Smith *et al.*, 1955).

The bats exposed to 15,000 r or more show signs of altered function of the nervous system beginning immediately after irradiation. These signs consist mainly of a high level of spontaneous activity (running and flying about in the cage), circus movements, and polydipsia. These activities are minimal after

15,000 r, but become more marked as the dosage is increased. To test the possible effects of polydipsia, groups of 6-10 bats were completely deprived of water after being exposed to 15,000-60,000 r and placed in a room at 5°C. These animals showed the same mortality patterns as irradiated bats which always had access to water when in the cold.

TABLE III

The Influence of X-irradiation upon Ability of the Bat to Enter Dormancy when Exposed to 5°C (January experiment) (Smith, 1959b)

X-ray dosage	Number of bats[1] in dormancy		
	day 1[2]	day 2	day 3
500 r	40	40	40
5,000 r	40	40	40
15,000 r	38	38	38
30,000 r	33	33	33
45,000 r	0	0	0
Control	40	40	40

[1] 40 bats per group except for the 60,000 r group which contained 12 bats.

[2] Time after X-irradiation and exposure to 5°C.

TABLE IV

The Influence of X-irradiation upon the Ability of the Ground Squirrel to Enter Hibernation upon Exposure to 5°C (Smith, 1959b)

X-ray dosage	Number animals	Number of ground squirrels in hibernation						
		day 1[1]	day 2	day 3	day 4	day 5	day 6	day 7
800 r	12	7	7	10	12	12	12	12
900 r	12	5	9	9	10	10	10	10
1,200 r	12	—	—	12	12	12	10	12
5,000 r	6	1	2	0	0	0	0	0[2]
10,000 r	6	1	1	0	0	0	0	0[2]
Control	70	35	65	67	67	67	67	67

[1] Time after X-irradiation and exposure to 5°C.

[2] Animals die on 8th and 9th day after irradiation.

Signs of heightened irritability and increased spontaneous activity are also seen in ground squirrels exposed to 5000 r or 10,000 r. These signs are apparent for several days after irradiation both in animals placed at 5°C and 23°C.

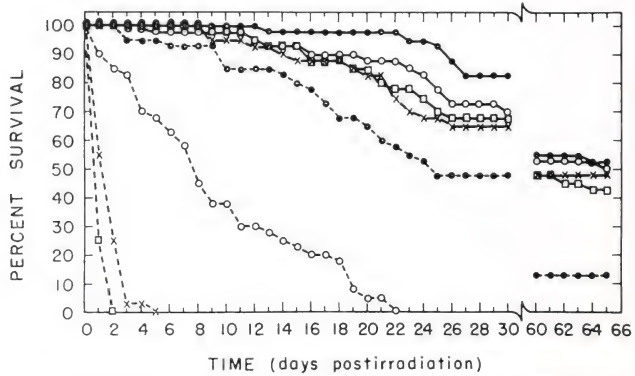

Fig. 1. The influence of X-radiation upon the mortality of bats in a 5°C environment (January experiment) ●———●, control; ○———○, 500 r; X———X, 1000 r; □———□, 5000 r; ●- - - -●, 15,000 r; ○- - - -○, 30,000 r; X- - - -X, 45,000 r; □- - - -□, 60,000 r. All groups contained 40 animals except for the one exposed to 60,000 r, which contained 12.

It may be of interest to note that the later in the winter that bats are collected and irradiated, the greater the percentage of animals that fail to become dormant within the first two days of exposure to cold (Fig. 2). Data similar to those shown for 30,000 r in Figure 2 were also obtained in a series of experiments with 40,000 r as the radiation dosage.

In yet another experiment, groups of 12 bats were exposed to 30,000 r in late March and kept at 23°C for 5, 10, or 24 hours before they were placed in a room at 5°C. All of these animals survived the stay at the higher temperature but died during the first 48 hours after being placed at 5°C.

The indications that irradiation can disrupt the maintenance of hibernation have been confirmed in other experiments on the bat (Smith and Thomson, 1959) and ground squirrel (Smith, 1959a). Prior to and early in the course of continuous x-irradiation, bats that are hibernating at 5°C have a rectal temperature

of about 5.5°C and an oxygen consumption of 0.03 ml/gm/hr. When a dosage of about 15,000 r has been reached, the rectal temperature and oxygen consumption begin to rise and reach values of 10-12°C and 5.0 ml/gm/hr, respectively. At this time the bats are awake and active. Ground squirrels that are exposed to 1000 r during hibernation at 5°C remain dormant during irradiation but become fully awake and active about one hour later. The animals stay awake for several hours and enter hibernation once again.

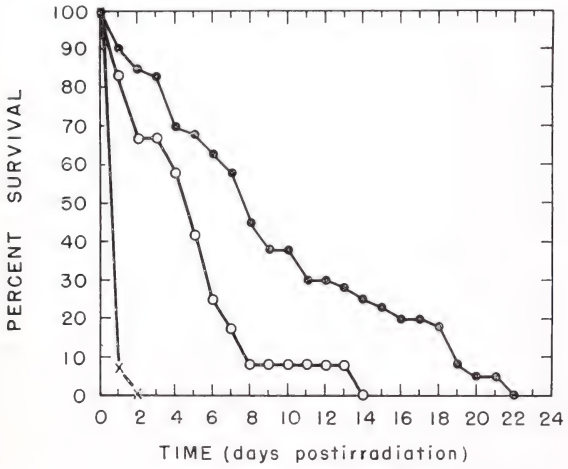

Fig. 2. The influence of time in hibernation upon mortality of irradiated bats in a 5°C environment. ●———●, collected and irradiated in January (40 bats); O———O, collected and irradiated in February (12 bats); X———X, collected and irradiated in March (13 bats). Radiation dosage 30,000 r.

The several experiments described above indicate that only those dosages of radiation that are high enough to elicit signs of altered function of the nervous system prevent the bat and the ground squirrel from entering hibernation. Animals exposed to such dosages show unusually great spontaneous activity, and appear unable to become quiet upon exposure to the cold. The ability to assume a quiescent state in a cold environment seems to be a primary requisite for entrance into dormancy.

The finding that massive dosages of x-radiation are necessary to inhibit hibernation in the ground squirrel does not agree with a previous report (Doull and DuBois, 1953) that such inhibition is elicited by a dosage of 800 r. Since both these and the above experiments were carried out on the same species collected in the same region of Illinois, it is difficult to account for the discrepancy in results except perhaps on the basis of a difference in nutritional status in the two sets of squirrels.

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DISCUSSION FOLLOWING SMITH'S PAPER

WIMSATT asked if adequate consideration had been taken of hypoplastic tissue conditions or relative state of activity during irradiation. SMITH replied that it had been considered and explained further that in the marmot there was no evidence of mitosis either in the controls or the hibernating animals, but that in ground squirrels there apparently were indications of low level mitotic activity in tissues in the hibernating state. WIMSATT then observed that a difference between recoveries of hibernating vs. non-hibernating animals after irradiation may be a function of differences in the states of their blood-forming tissues. In non-hibernating animals homoplastic differentiation may predominate in the active marrow, whereas in the hibernating marrow the blood cell formation could be pushed closer to the stem cells (heteroplastic differentiation). SMITH replied

that after irradiation one would expect the dividing cells to be much more sensitive and more easily destroyed, that in the active animal irradiation would be more destructive than in the resting marrow of hibernation; this is to be noted in the case of the marmot which shows no indication of cell damage during hibernation, but when removed from hibernation, radiation damage rapidly develops.

MENAKER asked if the remarkable longevity of bats could be correlated with an ability for unusual repair in the hibernating bat after radiation. SMITH replied that he knew of no evidence for or against this.

POPOVIC then showed a slide bearing on the viability of hibernating animals in a situation of oxygen poisoning. The data he gave showed that ground squirrels would live in a pure oxygen atmosphere at 6 atmospheres for 0.3, 7, and 13 hours when in the euthermic, artificially cooled, and hibernating states respectively (ref.: V. Popovic, R. Gerschman and D. Gilbert, unpublished observation). MENAKER asked how the results seen compared to the rat. POPOVIC replied they were just the same as in uncooled ground squirrels. SOUTH then indicated he did a similar probe experiment comparing rats and hamsters at high oxygen tensions, and found that the onset of convulsions occurred at the same time in both, but that rats died quickly thereafter, whereas hamsters survived as long as 6 hours after the onset of convulsions. He believed that this effect was probably due to a hypothermic condition, although he had not measured body temperature.

FOLK asked if SMITH had any information on the local effects of radiation of hibernating animals, such as possible protectiveness of hibernation against cataracts of the eyes following such radiation. SMITH replied that he did not.

STRUMWASSER then asked if a relationship existed between survival time following irradiation and the metabolic rate for the same species. SMITH replied that there are correlations in homoiotherms in experiments employing thyroid extract. Because of their long post-irradiation survival time, it might be thought that bats are extremely radio-resistant. If one considers that bats at rest have dropped their temperatures to the environment, one can account for the prolonged survival time on the basis of a lowered metabolism and rate of development of damage.

XXVII

PANEL DISCUSSION

Albert R. Dawe, Chairman
E. F. Adolph
George H. Bishop
Kenneth C. Fisher
Donald R. Griffin
Rev. Basile J. Luyet
C. Ladd Prosser

DAWE: There are a number of ways of beginning a discussion such as this. I have chosen the method of going from the general to the specific. I would like to go right to the heart of the matter rather than sidestep it. The initial question for the panel's consideration is: "Is 'hibernation' a valid term?" I begin this with the understanding that perhaps we are more or less like a group of people meeting in the 1500's and discussing "phlogiston" before the discovery of oxygen. Dr. Griffin.

GRIFFIN: I think it is a perfectly valid, useful term. I use it all the time. I think I know what it means, although I do think it is one that has to have a little qualifying. Obviously the hibernation of a bear is something very different from the hibernation of a turtle, but this mammalian hibernation, I think, has been operationally defined even in the last day or so and was probably further operationally defined the first day of the conference (which I wasn't able to attend). I think if you get into terminology and start rejecting well known terms, you will just have to call hibernation something else and that will make all the problems more serious.

PROSSER: I was impressed by Dr. Bartholomew's plea the other day. He indicated that, physiologically, hibernation and aestivation are not greatly different. His proposal that these terms be restricted to the changes which occur under natural conditions rather appeals to me. I'm not convinced that his suggestion of "facultative hypothermism" is really an adequate substitute, however. In a sense, it is "facultative poikilothermism" rather than hypothermism. I wonder whether the phenomena that we are discussing, which are certainly all-inclusive with respect to physiological processes, don't fall into something of a spectrum. Perhaps someone else will comment on this. Not

really having worked with hibernation myself, it is my impression that the differences among different hibernators and certainly the differences in the degree of torpidity are quantitative rather than qualitative. According to the time involved in the torpid reaction, the differences will become more marked. I wonder whether the overall syndrome isn't perhaps similar qualitatively for all these patterns but when one starts quantitating, differences become more marked.

DAWE: It would be unique, of course, for this group to announce to the scientific world that hibernation was *not* a valid term. We have had the question raised by Dr. Bartholomew, and I think all of us have been stumbling over it ever since that moment.

BISHOP: I think that the term "hibernation" limits your field if you take it strictly. If you take this thing as a means of saving energy, varying metabolism, then it is a very general term biologically. Thousands of plants do it. Many more animals take some precaution to conserve energy. For instance, an injured animal crawls off in the bushes and lies quietly. He cannot eat, so he stops activating himself; he is conserving energy again. There are all kinds of expedients that animals and plants take. This is quite a general biological proposition — if you take as a central problem of hibernation the "turning off of the heat," of the energy. This mammal is a special case because he has to have some particular kind of stimulus to do it and some particular expedient for getting out of it when he is in too long. I suppose those two things make it "hibernation" rather than just saving energy.

FISHER: By the same token then, Mr. Chairman, it would be necessary to start making some subdivision within the term. The enormous variety of manifestations included in the word "hibernation" has struck me most forcibly during these three days. If "hibernation" is retained as an inclusive term, confusion will result, it seems to me, if we do not clearly differentiate the various forms it may take.

PROSSER: Certainly "hibernation" applies – the word has been used for poikilotherms fully as extensively as for homoiotherms. We have not discussed it here as the broad general term. I still feel that Bartholomew has a very strong case for using this as a very general term and then limiting the specific.

BISHOP: Literally "hiber" means winter, and "hibernation" means passing the winter; this is the way the animal gets through a cold period. The term as we use it applies to a certain little corner of the study of the way animals and plants get around all kinds of stressful situations. You are limiting yourself by this definition to a certain narrow field, and if it led you to exclusive interest in that field I think it would be a pity.

DAWE: I gather that this group believes the word "hibernation" (the "winter sleep" or "winterschlafen") no longer covers all the things we talked about in the last 2½ days.

LUYET: The question: "Is hibernation a valid term?" calls, first, for a definition of the term "valid." Of the several meanings which may be attributed to "validity," I would like to examine shortly here the following three: (1) Has the term "hibernation" been "scientifically scrutinized" in such a manner that its introduction in a biological dictionary as an adequate designation of a phenomenon of nature can be recommended? (2) Is the term "hibernation" obviously misleading so that a substitution should be proposed? We have an example of such a situation in the terms "warm-blooded" and "cold-blooded" animals which were replaced, respectively, by "homoiotherms" and "poikilotherms." (3) Is the term free enough from evident ambiguity or obvious inexactitude that it may, without serious inconvenience, be retained for practical use? One may notice that improper terms are often used in common conversation, e.g. the "rising" of the sun. The answer to (1) is, obviously, "No." Even if we would like to scrutinize the nature of hibernation, our knowledge of facts about it does not seem to be advanced enough to permit a thorough analysis of that phenomenon. The answers to (2) and (3) would depend on the actual disclosure of evident ambiguities, obvious inexactitudes, or misleading connotations of the term "hibernation."

DAWE: The second question for the panel: "Did you gain the impression that there was sufficient distinction between hypothermia and hibernation to clearly separate the two phenomena?"

GRIFFIN: Let me have another try and see if I can make a more congenial comment. As I look back I see that this program as a whole is entitled "Natural Mammalian Hibernation" and I still think I know what that means, and I think operationally

(again) that I learned a lot about natural mammalian hibernation, although some of the papers have certainly dealt with rather "unnatural" situations. Just as a basis for discussion, I will stick my neck out here and say that what impressed me in the last few days was the difference between a natural hibernating state of an animal, in which it seems to be very cleverly regulating its physiological functions at several levels of temperature and several levels of activity, and some of these cases of unnatural cooling, in which the most striking difference, to me, has been the absence or the much lesser degree of this very regulation. So, as one who knows less and less about the more and more he has heard in the last few days, that stands out.

DAWE: You feel then that there is a distinction between these two phenomena. One should not confuse them; they are indeed distinct?

GRIFFIN: They are obviously going to merge and be a continuum but the distinction remains very real.

PROSSER: That's why I would raise the point of quantitative rather than qualitative differences. We have a whole spectrum of degrees of the hypothermic state. One comment I would like to add to a discussion of the day before yesterday. We talked about this matter of "turning the thermostat off." Some of us have used the term "setting the thermostat down" rather than "turning it off;" it seems to me that this is a little more appropriate, although I am not sure that either one is very strictly accurate.

ADOLPH: I would like to emphasize further the regulatory aspects involved in both hibernation and hypothermia. So often we think of hypothermia as a way of paralyzing an organism. It seems to me it is not just that; perhaps we are blocking a few processes but at the same time we are uncovering a host of other processes and regulations which weren't apparent before. This is the aspect of the subject which interests me particularly. As an example of the things we uncovered — who would predict that it is possible for the heart to continue beating to a certain temperature, for nerves to keep on conducting to another certain temperature and then come to a cutoff or "biological zero?" Now the significance of a "biological zero" is the same, perhaps, as the threshold for some activity. This is one of the characteristics of this activity. If we then go above the "biological zero" we find some sort of relation between an activity and that temperature, but the temperature coefficients (or whatever you wish

to call them) for the biological activity which have been measured in isolated tissues have in no case agreed with the temperature coefficients in the same activity in the intact organism. This, to me, illustrates the fact that there are factors at work influencing this activity, such as rate of heartbeat or rate of oxygen consumption, which were not apparent until we used hypothermia to uncover them. Hibernation, however, is another combination of regulatory relationships, and I don't expect to find very much in common between hibernation and hypothermia, because hibernation includes so much more than hypothermia.

LUYET: The question of the difference between hibernation and hypothermia is, of course, immediately related to the question of the difference between hibernators and non-hibernators. The following differences have been mentioned, in the course of this conference, as characterizing these two groups of animals: (1) a hibernator immersed in cold water, in the summer, cools more rapidly than a non-hibernator; (2) the lowest temperatures from which a hibernator and a non-hibernator recover are different; (3) the temperatures at which their metabolic activity stops upon cooling are different; (4) the energy consumption of a non-hibernator anesthetized by cold does not fall below that of its basal metabolism; that of a hibernator in hibernation falls to much lower values; (5) some physiological properties of the nerves, in particular, conductivity, are different in the two groups of animals; (6) so are some physiological properties of muscle; (7) so are some of the functions of the endocrine system; (8) the respiratory rates of brain slices indicate a different response to the same treatment; (9) the resistance to anoxia is different. Thus, definite characteristics justify the classification of mammals into two groups: hibernators and non-hibernators. But the existence of marked differences between them does not mean that the two groups should be considered as entirely distinct and self-exclusive. One may, similarly, enumerate a great number of marked differences between typical animals and typical plants; but this would not justify the conclusion that animality and "vegetality" are essentially different and self-exclusive entities.

FISHER: Would it not be better, Mr. Chairman, to say that hypothermia *might be a part* of hibernation. Hibernation is a larger term and involves more than what is ordinarily understood in hypothermia, not that they are entirely distinct but that one might be a lesser thing than the other.

PROSSER: Here is a quantitative difference again. When the bat or the hummingbird becomes quiescent the body temperature drops; this is just quantitatively different from the state of prolonged quiescence as in true hibernation.

GRIFFIN: Can I add to that? I think I agree with Dr. Prosser although I was using a little different wording. I would say there is a continuum here and in my favorite "beasty," the bat, a sort of intermediate situation between the poikilotherms and the most effective and resistant or stubborn homoiotherms. I suspect that there may be one aspect of the whole relationship of metabolism and temperature that needs further investigation, and I understand that the panel is supposed to think about directions or courses of action which may be taken in future research. I think of bats quite commonly as spending a great deal of their time in a rather poikilothermic state where their temperature falls to that of the surroundings, and yet I suspect they are still a part of the continuum with a very real homoiothermic phase of their lives, too. Now, to my knowledge, this hasn't been studied quantitatively. But when they are awake and flying they seem to regulate, as far as I can tell from casual observation. So you might think of bats ranging all the way from some of the more labile ones that are almost poikilothermic (but not quite because even they, when they are awake, may fly out in subfreezing temperatures and, I am sure, keep their body temperatures above 30°, probably above 35°, because bats don't seem to be able to fly below about 30°), to those which fly in a very hot place, in which case there is abundant evidence (H. Mislin, Helv. Physiol. et Pharmakol. Acta, **5**:C18, 1947; Rev. Suisse Zool., **48**:563, 1941; W. G. Reeder and R. B. Cowles, J. Mammal., **32**:389, 1951) that their wing membranes undergo considerable vasodilatation and vasoconstriction, so that I suspect they do regulate and are at least some sort of homoiotherm. Then, as various people have pointed out here and elsewhere, they can fight back against the cold and sometimes do to a limited extent. Dr. Wimsatt tells me that his favorite pets, the vampires, don't seem to fall to low temperatures but resist cooling. Perhaps he commented on that before I was here, so that I would just like to place these particular animals in Dr. Prosser's continuum, if I could.

PROSSER: May I ask a question, Dr. Griffin, in that connection? Do you consider that the hypothermia which a bat undergoes at rest diurnally is similar to or different from the state in the winter when it is more easily called "hibernating"?

GRIFFIN: I think the two states have a lot in common but there are some differences, too, and I would borrow your qualitative term and say I suspect some of the main differences between bats, when they do this either in winter or summer, and other mammals are in the time constants, i.e., they "turn this thermostat up and down" very quickly and flexibly. Superficially, they are much the same, in my limited experience, in summer and winter. If you put them in the cold they are quite likely to cool in either season. But, I have also noticed differences, and one of the most startling ones in my limited "philosopher's experience" is that if you do try to do this in summer, the bat usually dies in about a week. I do not think this is just lack of fat, because it occurs even when they are quite cool and they can't have burned up much fat or carbohydrate. On the other hand, of course, everyone knows that in the winter hibernation can go on for months. I suspect there are several adjustments to be made, and these are easier to make during the winter. Dr. Mayer described some of the histological changes.

PROSSER: Thank you.

DAWE: The next question for the panel is: "Were the lines of investigation described all-inclusive or were there glaring deficiencies in the research efforts represented?"

BISHOP: Would I be extreme in saying that about three-quarters of the work I've heard has dealt with the way the animal gets *out* of hibernation instead of how he gets *in*, or what "getting in" involves? This change of the muscle and so on, that enables the animal to function at a low temperature, and the arousal reaction which has been studied so much here are all secondary sequelae to the fact that he first has to "go down" and has to have some incentive for "going down." That's the first point.

DAWE: The technical problem of obtaining data on the animal going into hibernation is extremely difficult. In this respect, I think Dr. Lyman's paper was somewhat classical because he has obtained some data on induction. There was a smattering of other such information. Lack of data on induction represents more a question of technique and inability for technique to catch up with desire. I think all people working in hibernation would like to have data on induction.

GRIFFIN: Might I put in a plea for my friends the bats. If you insist on using stubborn animals like hamsters, of course it is difficult; but if you are working with bats it is extremely easy to study them while going into hibernation. They won't do it absolutely every time but they do it at least half the time that you want them to.

FISHER: May I then put in a plea, since this is a large problem, for the golden-mantled ground squirrel which in our experience can be subjected to high and low temperatures, to starvation and to platters of food, to water or a lack of it, to shortening days or lengthening days, or constant days or constant light or constant dark and still goes into hibernation when it is ready to do so in the fall, and similarly comes out in the spring. My point is that if both the bat and the squirrel are to be considered "hibernators," then we must be careful to realize that neither the ease with which the bat can be induced to enter hibernation nor, conversely, the striking difficulty of inducing hibernation in the squirrel during the summer months can be regarded as characteristic of hibernation in general.

BISHOP: Nor can aestivation be induced by subjection to a hot room.

FISHER: Exactly.

DAWE: Were there any other deficiencies in coverage of the hibernation problem noted at this symposium?

GRIFFIN: I don't see any deficiencies. I think it is a splendid coverage but I can see some things that I hope somebody will study in the future. I don't think these constitute a glaring deficiency but just real opportunities that perhaps are now ripe. I was very impressed by Dr. Strumwasser's work, and by the obvious importance of the nervous system in regulating affairs, and hope that he or someone will use similar approaches to a slightly different aspect, which I don't think his particular ground squirrel perhaps demonstrates as well as other hibernators — that is, this resistance to actual freezing. This phenomenon has been described a number of times — a hibernating mammal, as its body temperature gets too near the freezing point, fights back or generates a little more heat and actually regulates at this very low temperature level. I would like to know what the brain is "saying" in a bat or hamster or any animal that demonstrates this phenomenon at 0.5 degree, instead of 6.1

degrees. I would like further to reiterate the obvious that, if one is able to study the nervous system with implanted electrodes, as Strumwasser has demonstrated, one has tremendous advantages. He has certainly stimulated me even further than I had been stimulated by others before to learn how to do this. I can see a number of lines of work that I hope are going to come out of this.

LUYET: On the question of "lines for future investigation," I would suggest that the program of a future conference include: (1) Phenomena related to hibernation in lower animals (lower vertebrates and invertebrates), and in plants (in particular, dormancy and cold hardiness). (2) The development of hibernation in the course of evolution, and especially under the influence of changes in climatic conditions. A problem of great interest would be that of the origin and development of aestivation.

DAWE: In other words, a study of comparative hibernation in the first case and historical hibernation in the second.

ADOLPH: I think I would like to call attention to the desirability of listing the things that hibernators do *not* do, as well as listing the things that they *do* do. This is another way of describing what happens in a very profitable manner. For instance, I would make the generalization that no hibernator is so anxious to be cool that he uses evaporative cooling in order to get cool. Now this, to me, is a very significant thing. From an energetic point of view it might be cheaper to expend some water to get cooler than to have to spend more calories to stay warmer, and yet we don't know of any animal which uses this sort of refrigeration or gets cooler than his environment in this process of hibernation. However, behavior takes care of some of this, because animals (to some extent) seem to choose cool places in which to hibernate so that behavior is one of the elements in hibernation, no doubt. This, also, we haven't attempted to do much with. Of course, the whole matter of induction of hibernation is bound up with behavior as well as with physiological processes in the narrower sense. No doubt we all are looking for triggers which will induce hibernation and the fact that in 200 years we haven't found any could be discouraging to us, but if we now start to list all of the things that have been tried, really tried, and have failed, we might be farther along than if we simply wait for new ideas on things that haven't been tried before.

PROSSER: I have one comment on future lines of research. We're usually looking for cellular approaches and perhaps we could comment on that later, but I would like to recommend in this listing a little more use of the clinical method. It seems to me, from what I have heard here, that the hibernation phenomenon is a syndrome, something like the stress syndrome of Selye. There has been good use made of the clinical technique in approaching the stress syndrome by making as many different kinds of measurements as possible — physiological and biochemical measurements and, of course, histological — on the same organism. I can see great advantages in having a nearly complete clinical description of single organisms. I think that this has not been done as much as it might be. We attempt to make one kind of measurement and then hope to correlate that with another kind of measurement made by someone else.

GRIFFIN: When you say single organisms, you mean single species?

PROSSER: Yes, of course. But I do think that this clinical type description of a mammal might be useful. I see advantages in the cellular approach, also.

GRIFFIN: This is essentially what Benedict was trying to do in his day. In fact, this analogy occurred to me during the morning — that here we have this sort of "super-organism of investigators of hibernation" who bring in a whole variety of methods today and are not concentrating on one species of animal. But I must say that my own common failings make me quail at the thought of encompassing this encyclopedic battery of data.

PROSSER: Most of us like to work alone, but there is something to be said for group research. I have seen it succeed in this kind of problem, specifically with respect to the radiation syndrome. Sometimes, if you have a whole laboratory of people who are using different techniques on the same organisms they get a complete picture.

DAWE: Thank you. The next question: "What are your thoughts with respect to the 'thermostat'?"

BISHOP: You would expect this "thermostat" to be especially illustrated in plant reactions, if you remember how small a trigger is necessary to set off a plant hormone reaction. For

instance, a plant that has a day and night cycle which induces going to seed — that's a survival trait. Now a flash of light in the middle of the dark period — just one flash — breaks the cycle. Just a few seconds of light in a 12-hour period will change the whole pattern of that behavior in plants. I suppose there are things like that in hibernation, such as a cue to set this thermostat off, which may be so slight that you have not detected them as yet. Such a slight cue might, for instance, set up a hormonal reaction (as in some plants) which in turn could change the animal's behavior drastically. Now if you assume, as happens everywhere else, that these are hormonal reactions which control metabolic rates, then the essential thing here is a reduction of the energy. If you reduce the energy that an animal can put out, it doesn't matter whether or not he has a thermostat, he can't use it. Once you get the animal with the energy to come down below where he can keep himself warm, the thermostat is out. From there on, as I understand, most of the animals or many of them, follow the environment down.

GRIFFIN: No, I think the evidence presented is quite to the contrary. Before I came to this conference or heard otherwise of some of the evidence to be presented here, I would have agreed with you completely. But I think the data of Dr. Strumwasser and others have shown that there *is* some sort of thermostat, using the term loosely, that is, some kind of regulator.

BISHOP: Only at the beginning of induction when the animal hasn't gotten into the full hibernating state. Strumwasser's repeated warmings seem to be the persisting though sluggish functions of a normal or non-hibernating reaction. The only case where anybody demonstrated and insisted on regulation *at* a low temperature was in the bear and promptly the protest was made that he was not hibernating — that this is not hibernation.

PROSSER: Didn't Strumwasser find activity in the brain even when the animal was quiet and very cold?

BISHOP: Yes, sure.

GRIFFIN: Furthermore, I don't know whether he said this or whether it was in his paper that brain temperature seems to stay quite constant within 1/10 of a degree, at 6 degrees.

BISHOP: It does if the environment stays constant.

GRIFFIN: No, I gather with fluctuating environment. (Dr. Dawe then called on Dr. Strumwasser, in order to answer this point.)

STRUMWASSER: Well, I think you have missed one point and perhaps I can clarify this. If you remember, when these animals were dropping their temperature to certain critical points (the "test drops"), although the environment was relatively stable, plus or minus one-half a degree, each animal was regulating in the sense that it shivered and maintained a certain high level of brain temperature. The particular level of brain temperature was dependent on the number of days it had been in the cold. It was "seeking out a preferred level," let us say. I interpret these findings to mean that the biological system was choosing a level and regulating at that level.

BISHOP: I can make a different interpretation. I would say the animal is still sensitive to cold, and responds, as normally, by shivering but that his sensitivity to cold was changing, as my hand would adapt if I kept it in cold water. I wouldn't feel a cold stimulus as acutely. During hibernation the animal loses the reaction of shivering or other processes of heat production, and thereby cools off. At other times, cooling sets off energy-yielding reactions and thus prevents cooling, acts like a thermostat if you wish; the thermostat turns on the heat. Shivering is, in part, a reflex response to a sensory stimulus of cold, and probably, in part, to internal stimuli from low body temperature not resulting specifically in sensation. Strumwasser's evidence of repeated recovery of temperature, but each successive time from a colder state, appears to me to indicate that his animal is adapting. If the sense organs adapt, then shivering occurs, if at all, only to a more severe stimulation than normal. His animals appear to be cooling more rapidly than their cold receptors can become adapted. Perhaps if cooling were more gradual, the receptors would adapt progressively without these periodic recoveries. The question then would be: what permits the adaptation in the first place as compared to the non-hibernating state. Perhaps something happens internally, in the nervous system, let us say, which reduces the effect of cold stimulation but which can do so only slowly and in Strumwasser's case cooling as a stimulus overpowers cooling as a central depressant, and the temperature oscillates as a result. The "thermostat" then has at least two sections, a sensing apparatus and a means of increasing the heat, both processes being depressed by cold.

I wouldn't call that a thermostat, where the "stat" implies constancy. I am not defining a thermostat. I am defining a state of so little energy the animal never can get up to where the thermostat regulates, unless he can rouse out of hibernation completely.

GRIFFIN: There is regulation. I don't know whether the evidence was discussed here but there are several of these cases where down right near freezing, as an animal is cooled from 1 degree to 0.5 degree its metabolic rate increases, but it doesn't wake up . . .

BISHOP: You haven't got a thermostat actually but you have a reaction, perhaps to a further decrease of temperature, as a slightly effective stimulus.

GRIFFIN: Well, your cold reaction tends to regulate the body temperature at this very low level and this is what I mean by a thermostat.

STRUMWASSER: What is the thermostat? Can we come to a description of it? Is it not something which we put in a box for purposes of communication but which consists of detectors, tracts carrying information, integrations going on? We put all these parameters into a box and we call it a thermostat. We're not selecting any one area of the brain necessarily or just picking on a few neurons; it is a system. Don't you agree with that, Dr. Bishop?

BISHOP: Sure.

STRUMWASSER: Well then, this is brought into operation at these critical levels at which the animal's system chooses to regulate. If the external environmental temperature begins dropping, then the animal produces a little more heat and if the environment drops too fast, the animal tries to arouse after the thermostat has been activated, but the thermostat is never turned off.

BISHOP: No. That's what I was saying a while ago; the thermostat is there all the time but if the animal isn't producing enough heat, he can't use it. If you had a heater in a bath, a little heater, just enough to warm the bath a couple of degrees and if you set a thermostat at 10° above this, it would never act at all, but the bath would warm up a little above its surroundings.

DAWE: Next question: "Do you feel there was good evidence of an internal 'clock' mechanism in operation in hibernation in the mammal?"

FISHER: As I have already stated, the onset of hibernation in autumn and its cessation in spring in the golden-mantled ground squirrel are, in our experiments, independent of external conditions; this implies that they be controlled by such a "clock" mechanism. This question in relation to the other animals discussed here is still open, it seems to me. The arousals from hibernation which occur periodically every 4-5 days in the hamster, every 2 weeks in the squirrel, and so on, also suggest an internal "clock."

BISHOP: It seems to me you are getting into another semantic difficulty when you label these things "clocks," or when you call a reflex a "feedback" – using these mechanical terms to describe a biological process. Now I would say that it is a "cycle" . . .

FISHER: Agreed.

BISHOP: . . . a cycle of biological activity which recurs every so often and gives you a time interval. But you are getting a little bit — well, it's like an engineer talking about biology — he usually gets off on the wrong track.

GRIFFIN: You want to include the thought of the cycle being endogenous — whether you turn it off or not — the fact that it is not just regulated from the outside. That's the important thing.

BISHOP: There are plenty of recurrent phenomena in biology after all; they were invented before clocks were.

DAWE: Next question: "Did you feel there was good evidence for the presence of a hibernating gland?" (The panel all agreed that the influence of brown fat on hibernation had not been established.)

BISHOP: If you change the question a little bit and say, is there any evidence *against* a glandular control, I would say that there is no evidence against the pituitary. Nobody has "hung it on it" yet, but nobody has given it very thorough investigation.

PROSSER: It seems that the adrenal cortex is more involved than the pituitary. (Discussion of the adrenal cortex was postponed by Dr. Dawe.)

DAWE: Next question: "Should this conference go on record as advocating the point of view that further research of a crash-type into hibernation may make possible human hibernation which in turn may make man's space flight for long periods of time feasible?"

ADOLPH: I think the less said about space the better researchers we'll be.

DAWE: Next question: "Is it possible that, with prolonged hibernation, starvation ultimately results and the awakening signal is related to the depletion of food reserves, etc.?"

BISHOP: I asked somebody the question, suppose you carried an animal for an indefinite time at a cold temperature, beyond the normal winter. It would presumably wake up periodically and raise its temperature. Where would it finally die, at the cold level or the high level? This man thought it would die at the high level — it would come out sometime before it died at the cold level and get up to normal temperature and "burn itself out" there. Now, does anybody know?

FISHER: I don't think anybody, Mr. Chairman, during the two and a half days of this conference implied that there was a lack of either stored fat or carbohydrate at the end of hibernation.

BISHOP: But suppose you carried it out. Suppose you really put the animal under a continuous strain beyond that time. Where would it be?

GRIFFIN: I have occasionally left bats in a hibernating chamber that was reasonably good, and left them right on into the summer and they *do* eventually die, I think either of too little energy reserve, or possibly of desiccation, but I have no idea whether they awake first. Yet I sometimes think it is clear they die right in hibernation without waking up.

BISHOP: I imagine they might get exhausted and couldn't wake up, or might wake up and "burn themselves out" before they went back again.

GRIFFIN: You occasionally find them frozen into icicles in caves and I can't believe they let that happen if they were able to wake up at the last minute because it would only take a few inches of crawling to get away from the ice.

DAWE: It has been my experience that the animal arouses from hibernation periodically during the winter, irrespective of its fat stores. Of course, if fat stores are entirely depleted, it won't arouse. It dies in hibernation.

ADOLPH: I don't think we ought to limit this question to the matter of nutritional depletion, because there are other things which can give out besides the number of available calories. Presumably these deaths occur, as they do without any rewarming of the animal, from a large number of things, any one of which goes wrong or any number of which go wrong. So I don't think we can credit the end of endurance to any single sort of phenomenon in the animal.

BISHOP: That raises the question of what does really make him come out. Does he come out because he gets a little too cold or perhaps a degree below where he is safe? Is this a safety factor, coming out, or does he come out because of some cyclic activity which occurs and stimulates him or does he get some kind of external stimulus?

ADOLPH: Again I would form the opinion from what I have read and heard that there are a number of things that can figure in warming.

BISHOP: That is certainly one of them, isn't it: external stimulus. Poke him enough and he will come out, isn't that right? Another one, I understand, is abrupt cooling. Didn't somebody say that if you cool him rapidly when he is already down, he'll come out. I wonder if you cool him very slowly — sneaked up on him . . .

DAWE: He might not react.

BISHOP: He might not react because he was too cold to react to a weak stimulus.

DAWE: Yes, that's right. In other words, the Law of Dubois-Reymond may be operative. It's quite obvious, I think, that the deeply hibernating animal has certain sensitivities in this respect which are not too well understood. Next question: "Will the

panel compare the results they have heard from the various laboratories?''

PROSSER: They're all good.

DAWE: Next question: ''Does the evidence suggest that the heart during hibernation is 'independent' of the rest of the body?''

FISHER: How could the pressure be maintained, as it apparently is, if the heart were ''independent?''

PROSSER: Perhaps Dr. Adolph can speak on that.

ADOLPH: Well, our evidence was that the pulse rate in the intact hypothermic or hibernating individual has a different relation to temperature from that in the isolated heart. I don't know whether this is the sort of thing that is meant by ''independent.'' This is a very extreme form of independence, to have the heart cut out of the body and set up to beat by itself. There are various degrees of independence but I must say that very few of them have been studied in relation to hypothermia and hibernation. Why can't we test various degrees of ''independence,'' since there are identifiable connections between the heart and its environment which can be defined in terms of individual connectors. However, I don't think that this sort of research is as penetrating as lots of other forms because we'll probably find multiple ways in which the heart is related to its surroundings and the organism in which it is working. But, on the other hand, we have an opportunity in hypothermia, and perhaps in hibernation, to further define the relationships between the heart and the organism in which it works, which we don't have so obviously in the euthermic organism.

BISHOP: You might say that in many organisms, when the metabolism is under strain, the heart has a capacity of getting energy when other organs fail; the heart is one of the ''tough'' organs. Is that correct?

ADOLPH: We could define part of the relation between the heart and body in terms of energy-yielding materials. The mobilization of these materials must be of a reciprocal nature.

DAWE: Next question: ''A hibernator chilled to 6°C shortly dies, while an animal at 6°C in natural hibernation does not. What seems to be the most hopeful avenues of approach for solving this problem?''

ADOLPH: I have thought about this item a great deal, but I haven't any sovereign avenues of approach. It seems to me that the fact that a hibernator does periodically wake up, or warm up, means that there is some process which is in abeyance which has to be turned on or resumed periodically. I have tried to search for some examples of this in terms of electrolyte changes in tissues, but when you isolate the tissue to identify the change you have destroyed most of the situation in which the tissue worked. I think that it is true there is a longer survival of the hypothermic individual without rewarming in natural hibernation than there is after artificial cooling. I think this much has been established, and I think this gives us an opportunity to try to analyze what the factors are in natural hibernation which make the organism more tolerant of the low temperature. I think the answer is going to be a multiple one. I think it is going to come out in terms of the composition of the internal medium, in terms of the activities of the nervous system, in terms of the activities of the endocrine system, and a number of other factors.

GRIFFIN: That line of thought suggests to me an interesting point. While it is quite true that natural hibernators do wake up from time to time I think there are important exceptions. I think there are cases in bats at least, though they may be rare, where the same animal pretty clearly really did "stay put" for some months at a time probably without waking — though without having had a recording thermocouple, you can never be entirely sure. But this might be an important point for future investigation, to try to find and study more critically those cases, if they exist (and I think they do), where an animal really *does* remain for many days without any waking and to discover what is different about these animals and the conditions where this happens from the case where there is this periodic awakening.

BISHOP: It seems to me we have had several papers here defining this, more or less, which you could summarize under "general adaptation." For instance, nerve fibers are able to conduct at lower temperatures after they have been adapted in the body in the hibernating state. The heart, I presume, will work at a lower temperature and perhaps I would interpret Strumwasser's results as indicating a gradual adaptation to cold, so that the stimulating value of cold changes during the process of this cooling off. A "summer frog" and a "winter frog" are quite different. It is the same thing that was described here in

more detail in the measurement of nerve fibers. A "summer frog" nerve can be raised to a higher temperature before it is killed than a "winter frog's" can.

ADOLPH: I think I would like to qualify this idea about adaptation (with which I agree quite thoroughly in principle) in this respect: I think it is yet to be shown that you cannot get as much adaptation in the artificially cooled tissue as in the naturally cooled hibernating tissue, but I am not sure about this. A difference hasn't been demonstrated and its demonstration is limited by the fact that the artificially cooled animal doesn't last long enough at the low temperature to imitate the naturally hibernating one.

BISHOP: If you cooled him gradually enough and gave him time to adapt? Usually you plunge an animal into cold water and say, "Now he is cool." Well, the hibernating animal has a long preparation here and the preparation may not be due only to cooling, it may be a process going on here changing things in preparation for being cool.

ADOLPH: I think this factor of natural cooling alone has been imitated, so that the graded cooling was the same as in hibernation.

DAWE: I have only one more question for this panel: "Would the panel discuss the stress syndrome and the adrenal cortex in hibernation?"

BISHOP: You might ask in what sense hibernation is a stress or cooling is a stress. One might argue quite the opposite in a sense, since it relieves the animal of certain stresses. It becomes stressful only when the animal comes to the danger point of freezing to death.

DAWE: It doesn't freeze to death. Of course, in that sense it is not a stress.

GRIFFIN: Would it be too naive to put it the other way around and say the degree of cooling that is a stress for a non-hibernator is not a stress for a hibernator because of his adjustment and regulation.

DAWE: Would you expect this to affect the adrenal gland?

GRIFFIN: No, naively, I would guess that there would be less of the stress effect on adrenals on a hibernator going into

hibernation, in a normal situation. I don't know whether or not that is the case.

DAWE: Dr. Suomalainen, of course, has gone over this pretty carefully and I think he found that the stress syndrome was in effect. Is that correct Dr. Suomalainen?

SUOMALAINEN: Yes.

FISHER: It seems to me that there is a difficulty though, Mr. Chairman. Stress is an ill-defined word. When a rat that has been kept at 30°C is put at 5°C there is a "stress," if you like, that he cannot withstand and he dies. But if the animal is cooled gradually over a period of weeks, there is no longer the kind of stress that kills him. Do these two situations differ merely quantitatively? In the first instance the demand is beyond the physiological capabilities of the animal. In the second, the demand is adequately met.

GRIFFIN: Could I try to clarify that — you don't mean cooling a rat to a *body* temperature of 5°C, you mean environmental temperature?

FISHER: I'm sorry — in an *environment* of 5°C — yes, quite.

GRIFFIN: But supposing you do speak in terms of deep tissue temperatures. I never heard of anyone, except R. K. Andjus (J. Physiol., **128**:547, 1954), cooling rats to 5°C and I suspect that was a pretty stressful situation and this is just a more valid distinction.

BISHOP: That's the point — if you take an animal that insists on staying at 37°C and push him down, then he fights against it — that's a stress. I think anybody will agree that that is a stress. If this animal doesn't resist and manages to go down (slips down without effort), I don't see any stress about it at all. For instance, Strumwasser's animals may be reacting "stressfully" at first, and not finally.

FISHER: So you would rather not call this "stress" in a hibernator?

BISHOP: I would say the same process of cooling might be a stress in a homoiotherm and not in a hibernating animal.

PROSSER: And yet there are some clinical similarities.

FISHER: It depends upon one's definition of "stress," however. If any change that can be recognized in the adrenal is defined as the result of "stress" then any question is settled: hibernation is clearly a stress.

ADOLPH: Let's leave the adrenal out of the definition — why not? There must be many ways in which stress is expressed other than in the adrenal change.

BISHOP: You might say the process of hibernation is to avoid stress. After all, if an animal is going to starve to death or hustle for a living if he stays up at a normal temperature, he is avoiding that stressful existence by going into hibernation.

DAWE: I have no other questions for the panel. The panel now has an opportunity, individually, to express thoughts which they feel need expression. Dr. Fisher.

FISHER: This is probably out of order, Mr. Chairman, but I feel someone should express appreciation to the institutions and individuals who organized this meeting and made it possible to hold it under such pleasant circumstances. If it is appropriate I would like first to move a vote of thanks to them for these arrangements – and then we can get back to science.

DAWE: Thank you, Dr. Fisher.

FISHER: I like the point about the hypophysis mentioned by Dr. Bishop. Upon several occasions it has been noted that, as the body temperature of the hibernating animal goes down, its rate of metabolism falls somewhat more than would be expected on the basis of usual values of the Q_{10}. Now, removal of the hypophysis, in some mammals at least, lowers the metabolism of the whole animal and of its parts, if they be isolated for examination, by about 50 per cent. Could it be that the production of the hormone concerned with metabolic rate ceases during hibernation, thus causing a greater drop in metabolism than would be expected from the temperature change *per se?*

GRIFFIN: Has anyone tried to prevent animals that otherwise would go into hibernation from doing so by adding pituitary hormones — that would be the obvious experiment.

PROSSER: Didn't Dr. R. K. Meyer try that? (M. A. Foster, R. C. Foster and R. K. Meyer, Endocrinol., **24**:603, 1939).

GRIFFIN: Did it work?

PROSSER: As I recall, their controls weren't adequate.

BISHOP: There was a report — if you take out the pituitary, the animal still does go into hibernation but dies. Of course, they die younger anyway.

GRIFFIN: Well, that's hypophysectomy. Does this include the other — the addition of hormone?

FISHER: My remarks applied not to the mechanism causing onset and cessation of hibernation but instead only to the drop in metabolism which occurs as hibernation ensues. This drop seems greater than would be expected from the drop in body temperature assuming common values of Q_{10}. The situation to which I was drawing attention is exemplified by the aestivator. If I remember correctly, *Citellus mohavensis* allowed its body temperature to fall from 37° to 24° but its metabolism at 24° was only 1/20 of that at 37°. If the change in metabolism resulted solely from the drop in temperature, the unusually high Q_{10} of more than 10 would have to be assumed. By suspending all muscular activity, however, the metabolism of the animal in aestivation might be reduced by ½. If, in addition, the animal in aestivation is in this connection functionally hypophysectomized, its metabolism might be dropped because of this by another factor of ½. The drop remaining to be accounted for by temperature is now only 1/5 (not 1/20) and the Q_{10} required is only 3.5, a much more common value. Has anyone else given thought to the drop in metabolism in aestivation or hibernation?

GRIFFIN: Let's go back specifically to some measurements made by Hock (R. J. Hock, Biol. Bull., **101**:289, 1951) some years ago on metabolic rates of bats at different temperatures (all the way from 0.5 to 42°C). You could pick your Q_{10}, as I remember, anywhere from 2 to 5. But at different parts of the temperature range for this particular animal under these particular conditions you have a wide variety of Q_{10}s. So this in a sense reinforces what you were saying. But I extrapolated from your remarks to the working hypothesis that an animal goes into hibernation because of insufficiency in some one of the pituitary hormones. If this is so, you should get some confirmation by adding the hormone.

PROSSER: It seems to me that the alternative hypothesis would be to forget the pituitary and assume that this greater rate of reduction is due just to complete flaccidity and loss of all muscle tone. The active metabolism, in other words, is gone.

FISHER: Well, I think a lowering of half the metabolism might occur this way but no more.

GRIFFIN: At what temperature range?

FISHER: At 37° I would guess that the loss of all muscular tone would result in a loss of half the respiratory metabolism.

PROSSER: Somebody ought to try some of these things with animals that have been made inactive by curarizing agents.

BISHOP: Well, now if you are willing to go beyond the group of mammals which hibernate, there are plenty of cases of hormonal inhibition in metabolism: for instance, the grasshopper egg which demands a period of cold to remove that inhibition before it can go on growing. There is also a certain kind of a peach which grows until about June and then stops — about as big as your thumb — and waits for a month during the hot weather and then goes on growing again. But there is another peach, just like it otherwise, which keeps on growing and ripens about a month earlier. This is an aestivation phenomenon in the plant. What the rule is, I don't know — but something inhibits growth for about a month and then growth proceeds normally. When you get into the other reactions of plants, it is easy to see that there are all kinds of changes in metabolism factors which are probably hormonal, if you could detect the hormone and identify it, involving response to light and dark periods or to temperature changes and so on. One of the easiest things with which to change metabolism is a hormone, in plants certainly. It looks awfully suspicious here also. Now I suggest that it might be profitable to look not only at the hypophysis but also at the hypothalamus which is certainly a center where the cues of sensation might be registered — the reactions for heat, thirst, hunger, etc. as if the animal had sense organs, neural sense organs, there that recorded in the blood the presence of certain critical materials. This would make it a center from which you could get all kinds of influences over metabolic and other processes of the body. It would be a tricky job to do in a little ground squirrel — to take out some specific part of the thalamus or hypothalamus — but it might be worth doing.

DAWE: Dr. Adolph, do you have anything further?

ADOLPH: Could I add something to what has just been discussed about energy metabolism? Popovic has some evidence

that a hibernating animal has a very low energy metabolism and within five minutes can change to its expected metabolism without changing its body temperature (V. Popovic, Arch. Sci. Physiol., 11:29, 1957). In other words, this change is too sudden to get rid of a hormone, perhaps. It doesn't matter to me whether this agent is a hormone or not, but it is a very fast switch. It is comparable, in my estimation, to the switch from a resting to an active muscle: here you can step up the metabolism 50- or 100-fold with excitation. Hibernation acts in the reverse direction to step down the metabolism (perhaps not instantaneously but over an hour or less) after which high metabolism can again switch on within minutes. This, it appears to me, is the sort of control we have to look for; one which determines the total metabolism of an animal — not just the metabolism of one spot in the animal. It's not just the presence of more or less cytochrome C or something like that, but it's an activation of something that is all ready to be activated by some excitant which presumably spreads throughout the organism — that's the way I picture it.

FISHER: Muscle isolated from an hypophysectomized rat respires at about one-half the rate characteristic of muscle from normal rats. In some recent experiments we have found that the addition of lactate (and probably stimulation to contraction) raises the rate of each to the same value. Thus, hypophysectomy appears to lower the resting rate of metabolism without affecting the maximum rate possible. This situation fits what is known about respiratory metabolism during hibernation. Part of the drop in metabolism during hibernation must be accounted for by a process like hypophysectomy, which lowers metabolism independently of a temperature change and yet permits the maximum metabolism to be established very quickly as in an arousal.

DAWE: Anything further, Dr. Griffin?

GRIFFIN: I would like to add two thoughts. I will just call attention again to this extreme longevity in bats which puts them way out of line with other mammals of their size. It just happens that bats are numerous and various people started banding them enough years ago so that spectacular longevity records (like 21 years for a 7 gm species) have turned up not only in this country but in Europe. Dr. Eisentraut tells me that his bats are still being caught, just as mine are, and I think that it is as much a question of the longevity of the investigator as the longevity

of the animals. I wonder whether this may be shown also in other hibernating mammals. It would be much harder to get the data perhaps – but do ground squirrels have any greater longevity than closely related non-hibernating mammals? The obvious thought is, of course, that they are "burning their candles" only at one end or only at one corner and spreading out their quota of heart beats or units of metabolic energy. This might have some vague interest for people concerned with longevity. That's one thought. Thought two (suggested by the whole symposium, or as much of it as I have been present at, and particularly by Dr. Strumwasser's experiments) is one that I have already expressed this afternoon — that regulation, in part at least, by the central nervous system is, perhaps more than I ever realized before, the key to successful hibernation. And just to "freewheel" for a moment, how about somebody trying an experiment in which he takes an animal with a fairly plastic central nervous system that is not a hibernator, such as a rat, and tries to train that nervous system to regulate the internal affairs at lower and lower temperatures? Conceivably, this is the way to approach human hibernation, not by trying to find some "magic pill" but by trying to find out what it is that has to be readjusted and to approach this through the one way that in a general sense is well known — to readjust central nervous systems, namely learning. And with that final thought, I will pass.

DAWE: Dr. Bishop, do you have any final statement?

BISHOP: Nothing to add except my own experience, coming here as a complete "ignoramus," as to what hibernation is about. I have had to sort out 50 papers here and try to make some consecutive sense out of them, and I come up with something like this: This process of true hibernation in the mammal (that permits temperature to follow the environment) has three essential parts. The first is the inciting stimulus which is quite incidental, it seems to me, because it can be heat or dryness, or temperature lowering — any appropriate stimulus which the animal needs apparently sets off the same process. Second, the specific thing is the process of reducing metabolism. The third process is recovery from this situation. It will kill the animal if he can't recover; the animal is helpless unless he can come out of this state. Now a lot of the papers presented here described the various processes by which he comes out — which again however are more or less incidental. They are not the same for an animal

that hibernates at 30° as they are for an animal that hibernates at 5°. They may be different for other reasons, but again these are more or less adaptations to the environment. I, in my present view of the whole subject as a result of this conference, see the central problem here as the problem not of how he lowers his temperature but of how he lowers his metabolism. On one hand are the cues which any animal may get and which may be variable with the environment in which he lives, and on the other hand are the processes by which he recovers from a dangerous condition which he biologically prefers, rather than going on and starving to death at the lower temperature. Of these three parts, the central problem still is how the metabolism is reduced.

DAWE: Dr. Kayser may wish to speak of that later. Dr. Prosser, do you have anything further to add?

PROSSER: I would like to comment very briefly about one viewpoint which seems to me to have been omitted, although I missed the biochemical papers yesterday. No one has suggested that some of these metabolic changes may be analogous to enzyme induction phenomena. Granted that the phenomenon of hibernation involves many organs and that certainly in the final analysis one must study the organism as a whole, I would make a plea for more intensive fractionation — that is, a study of specific enzyme systems. I think that measuring oxygen consumption is just about as crude a method of studying biochemistry as counting the number of vertebrae and saying that this is studying morphology. Yet, we are basing most of our conclusions about energy on oxygen consumption of the whole organism. The important thing as far as energy is concerned (or an important measure of it) might be the P/O ratios of the mitochondria from certain critical tissues, and it seems to me that we might well do a little thinking about the possibility of alterations in metabolic pathways. We know that there are many pathways from substrates out to oxygen and some of these are cross-linked at intermediate steps. We also know that in many kinds of organisms (I am most familiar with poikilotherms in this respect) we can shift the pattern quantitatively so that one pathway is predominant over another in a given state of, let's say, temperature adaptation. The working hypothesis seems to be reasonably well supported that we can shift enzymatic pathways by slowing one pathway, by causing pile-up of the substrate of this pathway, and then these intermediates may serve as inducers for an alternate pathway. And we know that this kind

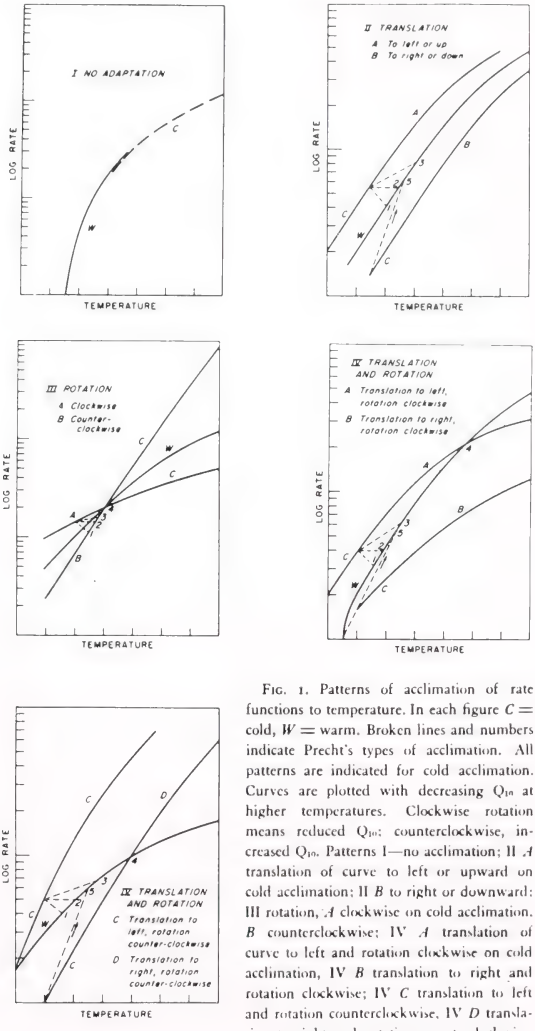

FIG. 1. Patterns of acclimation of rate functions to temperature. In each figure C = cold, W = warm. Broken lines and numbers indicate Precht's types of acclimation. All patterns are indicated for cold acclimation. Curves are plotted with decreasing Q_{10} at higher temperatures. Clockwise rotation means reduced Q_{10}; counterclockwise, increased Q_{10}. Patterns I—no acclimation; II *A* translation of curve to left or upward on cold acclimation; II *B* to right or downward; III rotation, *A* clockwise on cold acclimation, *B* counterclockwise; IV *A* translation of curve to left and rotation clockwise on cold acclimation, IV *B* translation to right and rotation clockwise; IV *C* translation to left and rotation counterclockwise, IV *D* translation to right and rotation counterclockwise.

(Reprinted by permission from *Physiological Adaptation*, C. L. Prosser, ed.; Washington, 1958, p. 173.)

of enzyme induction can go on not just with substrate (as microbiologists have shown) but with physical factors of the environment. We can bring this about in poikilotherms by changes in temperatures; we can bring this about by restricted oxygen. In hibernators we have both: reduced oxygen transport, and a lower temperature. It seems to me entirely possible that some of the enzyme systems are quantitatively changing in amount. I would like to see this general approach of more detailed enzymatic analysis, using inhibitors and purified enzyme systems, rather than just measurement of gross metabolism. Another bit of evidence is the one I just raised about the difference between active and resting metabolism. We know that in nerve and many other tissues the resting and active metabolism go by different pathways, at least in part, and this may very well play a role in the reduction of metabolism in hibernation. Now just one other point — I saw the curves that some of you had of oxygen consumption in relation to temperature, and there was some indication that there might be differences in the shape of these Q_{10} functions for non-hibernators and hibernators. I would suggest that one technique which might be used, in addition to the enzymatic one, might be to look a little more closely at the interpretation of these curves. In many temperature functions in poikilotherms we find that the curve can be shifted in either of two ways or in both of them. See Figure 1. In many instances there may be simple translation so that in an animal which is cold adapted the curve is shifted to the left of that of an animal which is warm adapted, and when measured at a given temperature the metabolism in cold adaptation is higher. Very frequently we may have a shift, in the sense of a rotation rather than a translation, and whether or not the rate of the cold adapted form is higher than that of the warm depends upon the temperature of measurement in relation to the point of intersection. The first shift can be interpreted in terms of a change in total enzyme activity. The second must be interpreted in terms of a qualitative change, perhaps a change in activation energy. This type of analysis can really be of use in trying to get down to the molecular level in tissue changes. Apparently some of you with whom I have spoken have not read the very beautiful work of Precht in Germany along this general line (H. Precht, J. Christophersen and H. Hensel, *Temperatur und Leben*, W. Berlin, 1955). I hope that another conference may emphasize the molecular approach to temperature adaptation including hibernation.

DAWE: Thank you Dr. Prosser. Do you have any final remarks, Dr. Luyet?

LUYET: Before attempting to answer questions such as that of the difference between hibernation and hypothermia, let us briefly glance at the development of our concepts, in general, taking the concept of hibernation as an example. One may distinguish three stages in the history of concepts.

1. *The "name giving" stage.* Primitive man, lost in a chaos of sense observations, and pressed for time by the necessities of material life, made distinctions hastily, often on a superficial basis, and assigned names to the things and actions that he had distinguished. Thus he developed the concepts of plants and animals, warm-blooded and cold-blooded animals, sleep and awakeness, winter and summer, daily sleep and prolonged lethargic sleep, etc. In the course of this observation of the world, he occasionally noticed that some animals remained asleep during the cold season; he called that phenomenon "winter-sleep" and did not think that there was much more in it to be concerned about. This development of concepts was the work of the budding scientist in primitive man, and coining words was his means of publishing the results of his observations.

2. *The inquiry stage.* Later, man, having more leisure, examined things more carefully. He developed instruments to improve and extend his observations: the microscope, the watch, the thermometer, electric meters, etc. He now measured the temperature in the bodies of animals and in their environment; he determined the time taken by an impulse to travel along a nerve, etc. Then he tried to fit his new observations in the old frames of concepts which were a part of his mental equipment. Numerous questions of the following type arose: Is a particular cell observed under the microscope an animal or a plant? Is a particular animal a warm-blooded or a cold-blooded one? Does it belong to the category of *true* hibernators or not? Is winter sleep a *true* sleep? (The night sleep in man is apparently considered as the true sleep.) Is the sleep produced by a drug a *true* sleep? Is the anesthesia caused by cold a *true* sleep? One should notice too that the inquiry stage, also, led to an enormous number of new frames, that is, of new distinctions and new concepts expressed in new words, such as homoiothermy, poikilothermy, heterothermy, stenothermy, high and low homoiothermy; obligatory, stubborn, indifferent and morbid homoiothermy, to mention a few.

3. *The semantic stage.* Man finally realized that when he had assigned names to things and actions — either in the primitive stage of his intellectual life or, later, in the inquiry stage — he had proceeded too hastily. He started classifying before having gathered the necessary information. As a result, his classifications do not hold. The philosophy expressed in common language, which is primitive man's philosophy, and that expressed in some of today's scientific terminology are both superficial. The picture of the world supplied by *thorough* and *critical* observations does not fit either in the old or in the new frame of words. Questions such as: "Is a particular organism an animal or a plant?" become senseless, since the frame 'animal-plant' has collapsed. In a similar manner, the term hypothermia — which has been introduced hurriedly because of the urgent need of a new term for practical purposes in scientific communication — is now left in our hands with the request that we kindly see what we can do with it.

Conclusions. It seems to me that the logical conclusions of what has been said are that: (1) When new terms, such as hypothermia, are proposed, for designating phenomena which differ in some manner from phenomena designated by old terms, such as hibernation, one could set as the primary criteria for accepting or maintaining these terms on a temporary basis, that they be free from obvious contradiction or ambiguity, and that they be practically useful in communication. (We should, of course, be aware of the limitations of these terms and of their temporary status.) (2) Questions imposed upon us by the accepted terminology, for instance, as to whether a particular phenomenon is hibernation or hypothermia, then, lose most of their significance. (3) The real problem, of course, is to learn the *facts* beyond the *words.* In the case at issue, it is to find out how the various physiological functions are interdigitated in the various animals, and by which process they became so entangled. The findings will probably be that typical hibernators and nonhibernators represent two extremes in a series of complexly combined biological activities. When the gigantic task of establishing the facts is sufficiently advanced to permit their coordination, the question of where to place the labels (the words) will be a relatively simple and a relatively unimportant one. This conference, it seems to me, has contributed greatly to the task of establishing and coordinating the facts and, therefore, to the progress of the science of hibernation, in its inquiry stage.

DAWE: Thank you, Dr. Luyet. Dr. Fisher.

FISHER: Dr. Prosser has noted that the data of several participants in this symposium indicate differences in Q_{10} functions between hibernators and non-hibernators, suggesting differences in the limiting metabolic pathways. This is reminiscent of the work on an isolated succinate oxidizing system studied by Z. Hadidian and H. Hoagland 20 years or more ago (J. Gen. Physiol., **23**:81, 1940). In those experiments the Q_{10} was changed by appropriate additions of cyanide and malonate, respectively, owing presumably to the ability of cyanide to make the cytochrome systems limiting and to the ability of malonate to make dehydrogenase activity limiting.

PROSSER: I think that you people who are working with hibernating animals ought not to hide your light under a bushel but to sell these tissues to the biochemists. It seems to me it would provide wonderful experimental material quite apart from understanding hibernation.

DAWE: Now I would like to open the discussion to the entire group.

XXVIII

GENERAL DISCUSSION

PENGELLEY: Perhaps I have some information on what Dr. Bishop was asking about, that is, what happens if you keep animals in the cold continuously after the hibernation period is over? I have done this with *Citellus lateralis* and there comes a time in the spring when they arouse from hibernation on a permanent basis; this does not seem to be correlated with the fact that they are running out of fat. This fits very nicely with Dr. Hock's work when he observed the exact time at which Arctic ground squirrels come out in spring and the time at which they go in, in the fall. If you do deny them food and water and still leave them in the cold, the period for which they hibernate will be prolonged, but they seem to decline rather quickly and eventually they die, and they die in hibernation in the sense that they don't come out and run around the cage frantically to get more food. They simply die in a hibernating state.

BRATTSTROM: I think Beer in Minnesota has studied this in the big brown bat (J. R. Beer and R. G. Richards, J. Mammal., **37**:31, 1956). If the winter in Minnesota is sufficiently long the bats will die in hibernation. During mild winters the bats have enough stored fat to last the winter.

GRIFFIN: Is he sure they didn't wake up at all; is he just finding them dead, or did he watch them?

BRATTSTROM: He watched them in routine intervals in caves, and I think in laboratory animals as well. I don't remember if he knows whether or not they arouse before death. I suspect that they did not.

DAWE: Are there other points to be discussed?

SMITH: I should like to extend Dr. Prosser's plea for a more detailed biochemistry of the hibernating animal to include also considerations of intra-molecular energy transfer and physical chemistry. Such considerations seem indicated in the case of data presented by Dr. South and by myself. With respect to the *in vitro* experiments of Dr. South, it should be possible to alter his systems with agents known to change the configuration of proteins. Such manipulations might give a clue to the explanation of his results, inasmuch as protein configuration (and thus

energy transfer along the protein or from protein to substrate) might be changed over the range of temperatures employed.

DAWE: Other points?

MENAKER: In the case of the relationship of artificially induced hypothermia to natural hibernation, it occurs to me that it should be possible to use artificially induced hypothermia as a tool to study hibernation itself, in this way: Dr. Strumwasser has suggested that in the California ground squirrel the animal may be "testing" his state of biochemical preparation for hibernation. Now it would be interesting, it seems to me, between these "test drops" to subject the animal to conditions that would induce artificial hypothermia and try to find out in this way something more about the state of biochemical preparation and also to do this at different times of the day. For instance, during his nightly natural temperature drop he may be less sensitive to some of the lethal effects of induced hypothermia.

HOCK: There may be a factor which is being overlooked in this discussion of hypothermia during hibernation. Hypothermia as we see it in the laboratory is accidental, but hibernation is a natural thing for which the animal is prepared or prepares. This is quite a large distinction and somehow seems to destroy the hope that we can use hypothermia in all ways that we might wish to use it to understand hibernation processes.

PROSSER: In other words, there is a difference between *acclimatization* and *acclimation*.

DAWE: Other points?

MORRISON: The question was raised, in fitting bats into our scheme of hibernators, as to whether or not bats regulated their temperature. Dr. Pearson described at least one example of a vespertilionid, I remember, that maintained a constant metabolic rate and wasn't forced into its natural diurnal cycle (throughout at least a 24-hour period). In the bent-winged bat *Miniopterus* (which was one of the three species I looked at in Australia), it is possible to show (under certain conditions when the animal was not flying) a *maintained level*. I think the idea has been advanced that temperature rise in the bat is sort of a tacit concomitant of muscular exercise — perhaps as in a bumblebee or a large fish as it warms up. These animals could maintain a fairly high level of temperature up to around 25° without

flying. Furthermore, under other conditions they seem to maintain a temperature around 30° (body temperature 30° and ambient temperature 20°) and I believe that when this level of 30° has been reached, either the animal is warming up from the cold or (in flying animals at a body temperature of 40°) is going down and regulating again at 30°. So it seems to be positive regulation and this is a rather significant level because this 30° figure is one which permits the animal to fly. Below that it cannot fly and above that it can fly. It may be a significant value in Dr. Hock's bears also. You saw that his values are just above 30° and this means the bear is in shape to fight you.

BARTHOLOMEW: The people here who have studied mammals outnumber those who have studied birds with regard to hibernation in a ratio of 50 to 1. I would like to call to your attention that some of the problems we are discussing are amenable to analysis through the use of hummingbirds which remain active for many hours at a stretch and almost certainly at high temperatures, and do have prolonged periods of temperature depression at night. In addition, one of the most common birds we see in the United States, the chimney swift, is a natural for somebody to investigate. At least two kinds of swifts become torpid, and somebody ought to put a screen over his chimney and catch them and see what they can do.

DAWE: I have some questions for general discussion. The first is: "Has anyone tested the threshold of arousal at different stages of the hibernation cycle for a given type of stimulation?" I certainly haven't — is there anyone here who has?

SMITH: We have irradiated bats collected at various times during the winter and found different responses as the season progressed. When we studied arousal from hibernation, we found that over the period from January through March increasing numbers of animals awoke from hibernation in response to a standard exposure to x-rays.

ADOLPH: A number of persons have reported that the interval between the temporary awakenings changes during the season; isn't this a partial answer?

DAWE: I think the person who submitted this question is looking for a standardized arousal procedure.

WIMSATT: J. DeWilde and P. J. Van Nieuwenhoven published a paper 2 or 3 years ago (Publ. Natuurhist. Gen. Limburg.

Reeks VII:51, 1954); they did extensive studies on what will stimulate arousal in hibernating bats. They were interested in the micro-climatology of caves. I know they used tickling with hairs. They finally reached the conclusion that hibernating bats were particularly sensitive to wind currents, and practically not at all to sound.

BISHOP: The threshold of arousal might be a measure of the depth of hibernation.

FOLK: We have observed that some bats remain at a totally different level of dormancy than others, for long periods. Within a colony you can characterize the individual animals staying at different levels. I don't know just how that ties in with this except that it was determined by a stimulus.

MORRISON: On this question, I don't have any quantitative data on stimuli but I think this is relevant: in experiments with *Muscardinus* this past winter, there was a very striking change in their sensitivity to stimuli. In about 8 to 10 hours, in a particular case, these animals would wake up with the slightest disturbance (in a metabolic chamber monitoring the oxygen consumption). On the other hand, when we wanted to conclude the experiment about 3 days later, it was not possible to make these animals arouse at all, even though we took the chamber out and banged it up and down on the water bath. We had to remove the animal and put it in a warm temperature before it would awake.

LANDAU: Was that ever a two-way change — did you ever find one that was unarousable one day and two days later was aroused by just being looked at?

MORRISON: No. This is the kind of experiment you might say was not a planned one.

DAWE: Next question: "What is the first sign of arousal – temperature rise, heart rate change, shivering, etc.? Is the arousal initiated by internal metabolic processes or external stimuli, that is, reaction to cold?"

LYMAN: I don't think I quite understand the question. Does this mean natural arousal or disturbed arousal?

BISHOP: Yes, natural arousal. What brings them back? Why do they arouse during a steady state of environmental temperature, steady state of the animal's temperature? Why do they occasionally arouse, what sets them off?

KAYSER: I think that external and internal factors intervene in the arousal: in a refrigerator regulated at ±1°C, a ground squirrel awakes every 6th day in October-November, and only every 25th day in December; later on, in January-February there is a regular decrease of the length of the phases of uninterrupted hibernation. The conditions of temperature, illumination and noise in the laboratory being constant through the whole hibernation period, the existence of an "internal clock" is evident. But if the refrigerator is regulated at ±0.1 or 0.2°C, the arousals are generally less frequent; so the temperature fluctuations also play a part. It may be, also, that the factors of illumination-darkness alternation intervene, but I have hardly studied them at all. Another evident factor is the nutritional one: if the animals are given some meat before hibernation, they enter more reluctantly into hibernation and awake more often, but the number of accidental deaths is reduced (experiments done with garden dormice). But the arousals during hibernation are normal phenomena, probably necessities. In field conditions, many hibernators hibernate in groups in their burrows. In my experiments (Arch. Sci. Physiol., **6**:193, 1952) on two European ground squirrels hibernating in two individual cages placed side by side in the same thermally and acoustically insulated chest, it clearly appeared that the arousal of one induced the arousal of the other. It is certain that in the field the constancy of external factors is not perfect, and that the long phases of uninterrupted hibernation which may be observed in the laboratory are often artificial and harmful. As a conclusion, internal factors are indisputable, but external factors also play a role in the determination of the frequency of arousals.

DAWE: Any other points for discussion?

STRUMWASSER: I would like to ask Dr. Bishop a question. Do you find it difficult to conceive of a brain at 6°C under constant external environmental conditions (let's assume that it's technically possible) initiating a spontaneous arousal?

BISHOP: I don't know why it shouldn't be able to.

STRUMWASSER: All right; well, I think that's responsible for some of the arousals.

BISHOP: It may be. However, I wondered just what Dr. Mayer was talking about — what, in natural hibernation, happened that might cause these periodic arousals. Animals break

through the inhibition or depression, or whatever it is, of the metabolism and start shivering again. Now, shivering is a natural response to cold. Suppose the temperature went down a little bit; would the animal shiver like a normal animal shivered because he got colder at a new level of adjustment, or doesn't the change in temperature do it at all — the shivering just happens from some other means?

STRUMWASSER: But they do "spontaneously" arouse from deep hibernation when the environmental temperature remains unchanged and they arouse in response to some internal alarm. After all, they do seem to arouse on a rigid kind of schedule at the start of hibernation, so there are obviously internal factors which are present and we do not need to always ask for external factors being necessary for these arousals.

BISHOP: There might be both, of course.

PEARSON: I am thinking, now, of the daily torpor that animals like hummingbirds and bats go in for — as I continue to say "physiologically" — in hibernation. Take bats that become torpid every day and keep them in a roost deep in caves, in constant darkness, away from sound, and thermostatic, and yet it is part of their ritual that every evening at the same time they awaken themselves and come out. This, I am sure, is spontaneous arousal from hibernation. Hummingbirds, in metabolism apparatus under water and at constant temperature, awaken before daybreak (in darkness) spontaneously. I think these cases of arousal are clearly spontaneous and Menaker here has his bats in constant environments giving a little rise of half a degree each day. All you have to do is magnify that and it is spontaneous arousal.

LUYET: But then this means only that we do not know, does it not? You said spontaneous — they arouse spontaneously. Now "spontaneous" means "with a cause you can not identify." It may be just a molecular motion some place in the system.

PEARSON: I'm thinking of a cause arising within the animal — an endogenous event.

FOLK: It is important to add to this picture the fact that hibernating 13-lined ground squirrels may awaken with intervals of one day, three days, up to (occasionally) two weeks, at an environmental temperature of 5 or 6°C.

MENAKER: If we don't talk about the periodic arousals that occur during hibernation, but simply the *last* arousal — at which point the animal leaves hibernation for good — it seems to me that Dr. Hock's data (obtained from field observations on ground squirrels) pointing at almost exactly the same date every year is very suggestive that there is a yearly internal rhythm, as is Mr. Pengelley's data on colonies of ground squirrels kept under constant conditions which awaken spontaneously and then cease hibernating.

DAWE: Dr. Frank Brown has observed and reported on annual, monthly, and diurnal rhythms as a general physiological phenomenon. He has not, however, made these observations on hibernating animals.

PROSSER: I think that it is entirely possible that there are "internal clocks," but I am not yet ready to say that there may not be extrinsic factors of which we are not aware which are having influences.

MENAKER: On April 21st every year?

PROSSER: Might very well be.

HOCK: This is 8 feet under the snow, by the way.

DAWE: Next question: "Would an animal which is ready to hibernate be prevented from doing so by disturbance or sensory stimulation, or would it finally hibernate anyway? I think it would finally hibernate anyway. Is there anybody who would object to that?

SOUTH: Yes, I think that this has come up a couple of times but I know that many of us have had a similar experience. For my own part, one of my temperature control rooms broke down so I moved over to a converted decompression tank which was much noisier. The thermal insulation probably wasn't too much different. It was much noisier, rattled, and conducted sounds much better. Temperatures were identical to the controls with excellent regulation in both tanks; but for the whole summer I didn't get a single hibernation — for 2½ or 3 months, I didn't get a single animal to go into hibernation in the noisy tank.

LYMAN: I'd like to reinforce Dr. South's observations and I think a lot depends on the animal, here. But certainly with hamsters – what were you using, hamsters?

SOUTH: Yes, hamsters.

LYMAN: I know that investigators who have come to our laboratory have asked why our hamsters seem to hibernate better than theirs do. It almost always turns out that they have some sort of a compressor that goes on and makes noise or there is a heat exchange mechanism that blows in. Our hibernaculum is next to the morgue. This is as quiet, I think, as you can get, and we get better hibernation.

LANDAU: I think there is another difference here because we have had some of our ground squirrels in the warm room where they were with dogs, cats, monkeys, etc. The temperature, as far as I know, was above 20° all the time and in the fall and winter we had (I think I observed over 40 times at least) over 18 different animals in a definitely torpid state. Now they certainly weren't in what you would call "deep hibernation" at a temperature of 20°, but I do think that if these animals are "ready" to hibernate they will let their temperatures go. However, I also think that the more distracting stimuli you have, the less likely they will be to do this.

SOUTH: Yes, we have a couple of pet Belding ground squirrels in our laboratory which, during this past winter, hibernated very frequently and usually, apparently, hibernate for about 24 to 36 hours and then wake up due to the distractions, but they were hibernating on and off all winter long.

HOCK: I think the whole difference here is the fact that the ground squirrels make cyclic preparation for hibernation and hamsters do not.

SOUTH: Right.

DAWE: The next question: "Would a mild anesthetic, a soporific only, promote hibernation in an animal ready to hibernate?"

SOUTH: We tried a lot of things. A couple of years ago, Jack Twente and I gave some hamsters chlorpromazine (unpublished observations). We put them in a cold room and about 50 per cent of them died and the rest became hypothermic; the next morning the latter were fine.

LANDAU: In this business of anesthetics I'd like to ask a question. We did a great many experiments in which we tried

to anesthetize and cool ground squirrels and we had a terrible time trying to anesthetize them because they were so inconsistent. The dose to which one animal would react had no effect on another animal or on the same animal on another day. Therefore, I don't know how one is ever going to give them a *light* anesthetic.

BISHOP: I would suspect that you were injecting the anesthetic in slightly different places in the different animals or in the same animal. Intrapleural or intravenous injections are preferable.

ZIMNY: In respect to Dr. South's findings, at one time I injected Serpasil. Serpasil was new and everybody was doing something with it. All the 13-lined ground squirrels did was to lose weight. They wouldn't eat. But they never did go into any stage of hibernation, torpor, or reduced body temperature. If anything, their dispositions became more violent than ever. That was the end of the Serpasil experiment.

PROSSER: Dr. Luyet has a comment on one of the previous questions.

LUYET: A question which arose in my mind in hearing this morning's report is that of the type of physiological reaction involved in the Auer's effect, that is, the effect of some magnesium compounds in bringing about a lethargic state. Is it merely a form of anesthesia?

POPOVIC: Dr. Kayser says that magnesium is not necessary.

BISHOP: Magnesium sulfate is a perfectly good anesthetic. We use it a lot.

LUYET: Is that merely anesthesia then?

BISHOP: As far as I know.

SUOMALAINEN: I have made such experiments. But when we injected magnesium solution into hedgehogs and put them into the ice-box, the condition of magnesium anesthesia was entirely unlike the condition of natural hibernation. In the magnesium anesthesia, the heat regulation of the animals was deranged so that the homoiothermic state was changed into a kind of poikilothermy. Metabolism diminished, the higher functions of the nervous system were paralyzed and motility disappeared. On the other hand, sensibility and muscular tonus, which are

preserved in natural hibernation, were greatly diminished. The animals were very limp. And, finally, the magnesium injection always produced strong hyperglycemia (magnesium diabetes) in contrast to the marked hypoglycemia found in hibernating hedgehogs.

PROSSER: Do the cold and magnesium work in the same direction?

SUOMALAINEN: No, they don't.

KAYSER: It has been known for a long time that magnesium has narcotizing properties. If magnesium is given during the summer, then in all hibernators and in all other animals narcosis and hypothermia obtain. But, like all other suppressions of the nervous system, the hibernators live 1 to 3 days, perhaps 4 days in deep hypothermia but without the normal body position characteristic of hibernation. Hamsters are dead after 2 days; ground squirrels survive 5 or 6 days in this state.

PROSSER: I have one more question. I would like to ask Dr. Mayer or Dr. Smith: you mentioned the other day that mitosis is pretty well interrupted, at least in the crypts or the bone marrow, during hibernation and then as the animals awake there is an increased burst of mitoses. Is there any evidence that animals in this situation (where there is a stimulation of mitosis) might get something that would even become a tumor, or go into an especially excessive production of white blood cells? I am interested in it from the point of view of control of mitosis.

SMITH: I know of no evidence concerning this, up to this point at any rate.

DAWE: Thank you. In my own behalf, in behalf of the Office of Naval Research, and in behalf of the American Institute of Biological Sciences, I have certainly appreciated the opportunity to assist at this symposium. I wish to extend special thanks to the panel and to the scientists who have come to us from Europe: Dr. Johansson, Dr. Sarajas, Dr. Kayser, Dr. Eisentraut and Dr. Suomalainen. I think that this has been an outstanding opportunity for all of us at last to get together and to talk over our mutual interests. Now, I think Dr. Lyman has some concluding remarks.

LYMAN: I didn't realize I was supposed to make any concluding remarks but I would like to thank, particularly, Dr. Dawe whose "brainchild" this conference was.

DAWE: Dr. Morrison was my major professor "way back when." He is responsible for my basic interest. But the idea for the conference itself originated in a discussion you and I had several years ago, Dr. Lyman, with Drs. Hoek, Brace and F. Smith.

LYMAN: I would also like to thank Captain Ruebush and the Office of Naval Research who made the symposium financially possible and last, but not least, I would like to thank Mrs. Winquist and the wonderful staff of Endicott House for taking such good care of us.

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